Development of a Thermal Desorption Gas Chromatography-Mass Spectrometry Analysis Method for Airborne Dichlorodiphenyltrichloroethane

by

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For Wendy, my wife, who made all this possible,

providing support and love along the way.

For Tana and Andrew, each equally my pride and joy.

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"Accurate and minute measurement seems to the non-scientific imagination, a less lofty and dignified work than looking for something new. But nearly all the grandest discoveries of science have been but the rewards of accurate measurement"

-William Thomson Kelvin

I would like to express my profound appreciation and gratitude to Dr. Philip Smith, for his mentorship and dedication to educating me on the importance of accurate measurement.

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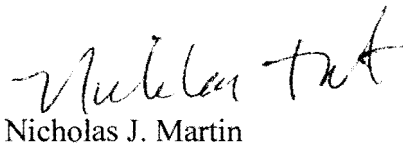

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ABSTRACT

Development of a Thermal Desorption Gas Chromatography-Mass Spectrometry Analysis Method for Airborne Dichlorodiphenyltrichloroethane

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Mosquito behavior assays have been used to evaluate vector control interventions to include the efficacy of spatial repellents (SR). Dichlorodiphenyltrichloroethane (DDT) is the only pesticide with SR activity approved by the World Health Organization (WHO) to control mosquitoes capable of disease transmission. Determining airborne DDT concentrations within assay systems is critical to understanding mosquito behavior following exposure to DDT. Current analytical methods are not optimized to determine short duration concentrations of airborne DDT during entomological evaluations. The goals of this project were to develop and validate a thermal desorption (TD) gas chromatography-mass spectrometry (GC-MS) method to determine the concentration of airborne DDT in mosquito behavior assay systems. Precision (relative standard deviation for each calibration point (0.8-9.0), linearity ($R^2 = 0.99$), and apparent recovery ($R' = 96.5\%$) were determined from TD GC-MS analyses of sampling tubes spiked with 1 to

250 ng DDT. Sample recovery with and without air sampling was 97.3% and 90.3%, respectively. During evaluation of a laboratory mosquito behavior assay system, 1 L air samples were collected over 10 min intervals. Significantly higher levels of airborne DDT were measured in the chamber containing DDT-treated textiles compared to chambers free of DDT. In the field, 57 samples were collected from experimental huts with and without DDT for onsite analysis. The concentrations of airborne DDT in the two huts containing DDT over a four day period with variable ambient temperature were 0.74 $\mu g/m^3$ (n = 17; SD = 0.45) and 1.42 $\mu g/m^3$ (n = 30; SD = 0.96). The results demonstrate that the TD GC-MS method was precise, reproducible, and linear over the span of 1-250 ng DDT. Furthermore, laboratory and field experiments utilizing this method confirmed that significant DDT concentration differences existed among treated and untreated spaces, permitting valid mosquito deterrent evaluation when comparing the two conditions. This TD GC-MS method addressed a need to measure short-term ($\leq$ 1 h) DDT concentrations in small volume (<100 L) air samples. Future studies should focus on identifying the lowest concentration of SR compounds, including DDT and pyrethroids, needed to modify disease transmission cycles through mosquito behavior.

Table of Contents

LIST OF TABLES

LIST OF FIGURES

LIST OF EQUATIONS

LIST OF ABBREVIATIONS

AI – active ingredient
ANOVA – analysis of variance
AUC – area under the curve
cm – centimeter(s)
DDCO – dichlorobenzophenone
DDD – dichlorodiphenyldichloroethane
DDE – dichlorodiphenyldichloroethylene
DDMS – 1-chloro-2,2-bis(p-chlorophenyl)-ethane
DDMU – 1-chloro-2,2-bis(p-chlorophenyl)-ethylene
DDNS – 2,2-bis(p-chlorophenyl)-ethane
DDNU – 2,2-bis(p-chlorophenyl)-ethylene
DDT – dichlorodiphenyltrichloroethane
DENV – dengue virus
DF – dengue fever
DHF – dengue hemorrhagic fever
DSS – dengue shock syndrome
d_f – film thickness
EI – electron ionization
EIC – extracted ion chromatogram(s)
EPA – United States Environmental Protection Agency
eV – electron volt(s)
GC – gas chromatography
h – hour(s)
i.d. – internal diameter
IRS – indoor residual spraying
L – liter(s)
LLIN – long lasting insecticide-treated net
LTM – low thermal mass
MS – mass spectrometry
mg – milligram(s)
min – minute(s)
m – meter(s)
mm – millimeter(s)
m/z – mass to charge ratio
ng – nanogram(s)
psi – pounds per square inch
R´ – apparent recovery
RNA – ribonucleic acid
RSD – relative standard deviation
s – second(s)
SIM – selected ion monitoring
SIM – simultaneous selected ion monitoring
SR – spatial repellent

TD – thermal desorption
TWA – time weighted average
UHP – ultra high purity
WHO – World Health Organization
YF – Yellow Fever

CHAPTER 1: INTRODUCTION

1. VECTOR-BORNE DISEASES

The World Health Organization (WHO) estimates that vector-borne diseases account for 16.7% and 13.0% of the global infectious disease burden and mortality, respectively (81). Disease vectors are organisms, typically hematophagous (capable of taking a blood meal) arthropods that transmit disease between organisms. The risk of acquiring vector-borne diseases is greatest in tropical and sub-tropical regions of the Americas, Africa and Asia (82) (Figure 1-1) where environmental conditions support vector populations. Of the many vectors known to transmit human diseases, mosquitoes are the most significant in terms of morbidity and mortality (39). There are more than 2.5 billion people living in close proximity of mosquitoes that can transmit a list of diseases including malaria, dengue, West Nile virus, Japanese encephalitis, and yellow fever (YF) (28; 29; 81; 92). Each year there are as many as 500 million cases and 1.3 million deaths due to mosquito-borne diseases (81; 87; 92). Today, the two most significant vector-borne diseases, in terms of morbidity and mortality, are malaria and dengue.

1.1 Malaria

Malaria is responsible for the highest burden of vector-borne disease worldwide; in 2010 the WHO reported 216 million cases and 660,000 deaths (most current data available), predominantly in sub-Saharan Africa (94). The reported cases may not represent the true burden of disease and some reports estimate there may up to 500 million malaria cases resulting in more than one million deaths annually (3; 81). A

parasitic infection, malaria is spread by mosquitoes infected with a protozoan from the genus *Plasmodium.*

The early stages of disease typically start 7 – 30 days following the bite of an infected *Anopheles* mosquito are characterized by flu-like symptoms: fever, headache, chills, and vomiting. The most severe form of malaria is characterized by severe anemia, coma, convulsions, and potentially fatal cerebral malaria (48). The case fatality rate is typically 5-15% in patients receiving drug therapy, but can approach 100% in untreated individuals. Infection with one of the four *Plasmodium* species known to cause malaria in humans, *P. falciprum*, *P. vivax*, *P. malariae*, and *P. ovale,* does not confer lifelong immunity and re-infection is possible (48).

1.2 Dengue

The number of dengue fever (DF) cases reported annually to the WHO has increased from less than 1,000 in the early 1950s to 2.38 million in 2010 (last year with complete data). Of the cases reported in 2010 more than 4,000 were fatal (97). The reported cases are likely an underestimate of the true burden of disease. Some reports estimate there may be as many as 50-100 million infections annually, with up to 500,000 cases progressing to the life-threatening dengue shock syndrome (DSS) and dengue hemorrhagic fever (DHF) (28; 29; 83).

There are four distinct serotypes of the dengue virus (DENV 1 - 4); a single stranded RNA virus from the family Flaviviridae, transmitted by the female mosquitoes from the genus *Aedes*. Infection with DENV can result in no signs or symptoms of clinical disease, mild disease characterized by mild to severe fever, headache, pain behind the eyes, and joint pain or life-threatening DHF fatal in up to 20% of cases

without supportive hospital-based care (83). Infection with one of the four serotypes is thought to provide lifelong protection against re-infection with that serotype. There is evidence that subsequent infection with a different (heterologous) serotype increases the risk of developing the more serious DSS and DHF.

2. Prevention and Control of Mosquito-borne Diseases

The range of the vectors for malaria and dengue, *Anopheles* and *Aedes* mosquitoes, respectively, includes all inhabited continents with only Antarctica free of mosquitoes (Figures 1-2 and 1-3) (89). The mosquito lifecycle and disease transmission are influenced by temperature and the presence of water sources, with seasonal transmission reported in temperate climates and yearlong transmission possible in tropical regions (42). The disease transmission cycle is similar for malaria and dengue; both pathogens are spread between human hosts when a female mosquito feeds on an infected person, then feeds again, transmitting the pathogen to an uninfected human host. Female mosquitoes, with mouth parts capable of taking a blood meal, require protein from blood for egg laying. The selection of where to lay eggs is highly variable, but *Anopheles* and *Aedes* species, like all mosquito species, require a water source to complete larval and pupal stages.

The prevention and control of mosquito-borne disease has focused on disrupting the human-vector-pathogen transmission cycle. This cycle is unique for each vector, often varying by region, and can include non-human reservoirs and host, complicating the development of mosquito-borne disease prevention and control strategies. Historically, successful control programs have focused on: 1) control of mosquitoes in larval and/or pupal stages, through elimination of breeding sites, e.g., draining swamps, marshes and

man-made water containers (22; 32), 2) modification of human-host susceptibility with chemo-prophylaxis or vaccination (87), and 3) reduction of adult mosquito populations through application of pesticides, e.g., indoor residual spraying (IRS)(84; 85; 87; 90).

While the relationship between man, environment, mosquito and disease was not described until the late 19th century, there are historic accounts of the association between swamps and fever. Without fully understanding the role mosquitoes played in spreading disease, swamps were drained near settlements, which removed mosquito breeding sites and reduced the vector population (32). The practice of environment modification was continued through the early 20th century, most notably during the completion of the Panama Canal. Extensive environmental modification was expensive and with the advent of novel drugs (65), vaccines (23; 31) and pesticides (51) during the first half of the 20th century, new global strategies were developed that focused on altering host susceptibility with vaccines and the control of vector populations with pesticides and repellents.

2.1 Drug and Vaccine Therapy

Another strategy to control or prevent vector-borne disease transmission is through the modification of human host susceptibility with drug therapy or vaccine administration. Drug therapy for malaria has been widely available since the introduction of the first synthetic anti-malarial compound, Atebrin®, in 1932, but resistance to these therapies has been described (65). At the time of this report there were no drug therapies available for dengue. Vaccines for both diseases are at different stages of clinical evaluation (66; 75); however, there are no licensed vaccines for either malaria or dengue despite concerted efforts by public and private partners (14; 73). Malaria vaccine

development is hampered by the lack of sterile immunity observed following natural infection; parasitemia is still observed in previously infected individuals. However, past malaria infections reduce the risk of clinical and severe disease (48). In contrast, natural infection with one of the four DENV serotypes confers lifelong protection against subsequent homologous infection. Epidemiological data suggests that individuals are at increased risk of developing DHF during subsequent infection with a heterologous serotype (35). The challenge in dengue vaccine development is the need to induce immunity to all four serotypes simultaneously to reduce the potential for increased risk of DHF.

The development of a vaccine against malaria or dengue likely will not herald the end of either disease. Outbreaks of YF, another mosquito-borne disease, were reported in Africa as late as the beginning of 2013, despite the fact that a highly effective vaccine is available (93; 95). Developed in the 1930s, YF vaccines were widely used in endemic areas through the 1960s prior to reductions in funding of vaccination programs. The YF vaccine is a live attenuated vaccine capable of inducing a protective immune response in 95% of people one week post administration (64). Despite the simplicity of the vaccine administration (single dose) and demonstrated effectiveness, the WHO estimates there are 200,000 YF cases per year (93). This suggests that a highly effective dengue vaccine will not be sufficient, by itself, to eliminate the risk of malaria or dengue in endemic regions. Compounding the concerns regarding the effectiveness of the vaccine to disrupt the transmission cycles of malaria and dengue, there is a risk of unintended serious adverse events following administration of vaccines. Serious adverse events, neurotopic and viscerotopic similar to natural YF infection, have been reported following

administration of the YF vaccine (74). Concerns related to the efficacy and safety of the YF vaccine underscore the need to develop and evaluate other methods to control the transmission of mosquito-borne diseases.

2.2 Pesticides-Indoor Residual Spraying

Control of disease vector populations is another method to disrupt the malaria and dengue transmission cycles. Many *Anopheles* and *Aedes* species are both endophagic and endophilic, meaning they have adapted to living near humans and feed and rest indoors part of or all of the time. Public health strategies developed to control mosquitoes have focused on placing pesticides inside homes, in close proximity to feeding or resting mosquitoes (44; 84-86; 91). Two of the primary strategies to control mosquito-borne diseases recommended by the WHO are the use of long-lasting insecticide treated nets (LLINs) and IRS to reduce exposure to mosquitoes (85; 91). In 2010, the United Nations estimated that IRS operations had protected approximately 75 million people at risk to malaria worldwide (88). However, the chemicals currently available for LLINs and IRS total 12 and are limited to four chemical classes (Table 1-1) (85). During IRS operations, insecticide is sprayed onto the interior surfaces of homes and interacts with vectors through mosquito contact with treated surfaces or volatilized chemical.

One of the most notable chemicals developed was dichlorodiphenyltrichloroethane (DDT). In 1939 Swiss chemist Paul Hermann Müeller discovered DDT's insecticidal activity, a finding that earned him the 1948 Nobel Prize for Medicine and Physiology "for his discovery of the high efficiency of DDT as a contact poison against several arthropods" (51). DDT was widely used for the control of malaria, YF (the same vector transmits DENV and YF), and sleeping sickness vectors

following World War II. Worldwide production peaked in the early 1960s at 400,000 tons annually (34) and in the United States production peaked in 1963 at 80,000 tons, but decreased significantly after restrictions were implemented in 1969 (21). It is estimated that two million tons of DDT were used in the United States after World War II, mostly for control of agricultural insects (21).

Dichlorodiphenyltrichloroethane is the most prominent member of a group of six related organochlorine pesticides (Figure 1-4). DDT's primary mechanism of action for acute toxicity is the impairment of nerve impulse conduction by opening sodium channels in neurons present in mammals and insects (16). The effects of acute toxicity range from altered sensations to convulsions and can lead to death from respiratory failure. Despite the widespread use and lack of controls during early application, however, no human death has been attributed to exposure to acutely toxic doses of DDT (21).

Exposure to DDT has been associated with increased incidents of cancer and has resulted in its classification as a probable human carcinogen by the United States Environmental Protection Agency (EPA) (21) and as a possible carcinogen by the International Agency for Research on Cancer (IARC) (34). Investigators have reported an association between exposure to DDT and cancers of the liver in laboratory animals, as well as breast cancer cases in humans (21; 34). Concerns over the use of DDT stem from the potentially long environmental half-life and the observed bio-accumulation and bio-amplification in the food chain (45). As DDT concentrations increase in higher level consumers, and it accumulates in adipose tissue, there is an increased and prolonged internal exposure to DDT and its metabolites. Due to the potential to cause cancer and accumulate in organisms and the environment, the use of DDT is restricted to control of

insects that threaten the health of the public under Annex B of the Stockholm Convention (76). Between 2003 and 2007 annual DDT usage is estimated to be in excess of 4,000 tons in 21 countries, with the majority of the DDT being used for vector control in India (5). Despite the restrictions placed on the production and use of DDT, it continues to be used due to its efficacy and low cost.

The fate and transport of DDT in the environment can be predicted based on its physico-chemical properties (Table 1-2). A white powder that melts at 109ºC, 4, 4′ DDT is relatively lipophilic (water solubility: 0.025 mg/L at 25ºC and log K_{OW}: 6.9), partitioning in organic materials such as soil and lipid rich tissues within organisms. Due to its non-polar structure and lipophilicity, 4, 4′ DDT does not typically partition into or accumulate in water. During agricultural pest control operations, DDT was applied to fields where it absorbed to non-polar organic components. Environmental half-lives of 100 and 150 days were reported following application to dry soil fields in Africa (10; 68) and the desert southwest of the United States, respectively. These relatively short half-lives are in contrast to the >20 years reported in soil collected in the northern United States (15) and Canada (37). This variation in half-life in soil is a function of microbial de-chlorination of 4, 4′ DDT resulting in the formation of 2, 4′ and 4, 4′ dichlorodiphenylethylene (DDE), de-chlorination related to photo-degradation (12; 24; 41; 55) (Figure 5), and temperature dependent sublimation into the air (10; 68; 70; 79; 80). In the air, 4, 4′ DDT can undergo photo-oxidation or remain unchanged, travelling long distances from the application site to deposit in areas that have not been directly exposed to DDT.

Semi-volatile compounds, like DDT, become airborne as either suspended solids, often bound to organic materials, or as chemical vapor (25). Despite being a solid at room temperature (20°C -30°C), 4, 4′ DDT has a measureable vapor pressure (the pressure exerted by the gas phase of a specific chemical at equilibrium with the solid phase) at temperatures as low as 10°C (78). Volatilization is an important process when describing the loss of 4, 4′ DDT from treated surfaces, e.g., agricultural fields (70; 79; 80) and following IRS operations (67; 77). Measureable concentrations of airborne DDT have been reported following treatment of glass (57), soil (30; 70), and inside test houses following IRS operations (67; 77). These studies suggest that DDT becomes airborne following IRS operations creating a scenario where mosquitoes could be exposed to DDT without direct contact with treated surfaces.

The effect on mosquito behavior following exposure to airborne DDT is not well characterized. Smith and Webley proposed that sub-lethal exposure to airborne DDT could elicit a deterrent response in mosquitoes; that is, mosquitoes exited or avoided entry into experimental huts following IRS with DDT (69). The authors noted that mosquitoes exiting the treated spaces had a higher survival rate, despite exposure to DDT confirmed by GC-electron capture detector analysis of ground-up mosquitoes in isohexane. Additionally, the level of DDT exposure (mean DDT mass per mosquito) was different between the mosquitoes within the house ($\geq$ 7 ng) and those that exited the space (1.5 ng). These results indicate that sub-lethal exposure to DDT had an excito-repellency effect which altered the mosquitoes' behavior (increased exit of treated spaces).

In an effort to better understand the excito-repellency action of DDT and the potential to modify mosquito behavior (1; 2), entomological assays have been developed to describe specific vector response following exposure to airborne DDT (9; 26; 96). These include both laboratory and field test systems that measure repellency (i.e., deterrence or reduction in mosquito entry), irritancy (increased exit), and mortality (8; 9; 13; 60; 61). The spatial repellent (SR) effect of DDT has been the focus of behavioral evaluations with the malaria and dengue vectors *Anopheles* spp. and *Aedes* spp., respectively (27; 63; 72). Combined, these studies demonstrate that DDT elicits SR activity in mosquito vectors (90).

At the time the studies mentioned previously were conducted, there were no published analytical methods to measure the concentration of airborne DDT over short sampling intervals (≤ 1.0 h). Therefore, the concentration of DDT relevant to SR activity/mosquito behavioral response in test systems could not be determined with temporal resolution. Although defining the short-duration concentration of airborne DDT was not a specific objective of previous evaluations, it is now recognized as a critical component in the development of novel vector control strategies. This is because an understanding of the specific conditions required to generate sufficient airborne concentrations of a SR chemical, e.g., DDT, to repel mosquitoes will allow identification of operationally significant parameters relevant to SR control strategies. These parameters include product format, placement in a given space (i.e., home), required DDT loading levels to elicit minimum thresholds of mosquito responses, effective distance, and environmental conditions such as temperature, humidity, and wind speed, that may affect airborne SR concentrations.

2.3 Air Sampling

Quantifying the concentrations of airborne SR chemicals during mosquito behavior studies is critical to understanding the relationship between chemical exposure and mosquito behavior. Such information can be used, in part, to establish potential entomological correlations with health outcomes, such as percent reduction of mosquito entry and/or frequency of biting rates relative to the frequency of mosquito-borne disease. Standard approaches have been developed to characterize the efficacy of SR compounds and strategies on mosquito behavior and health (96). Various approaches have been used to measure pesticides in air (7; 40; 49; 50), including the use of standard methods developed by the EPA (54; 67). Traditional sampling and analysis methods can be divided into four stages: sample collection, sample preparation, separation, and quantitation (53). Sample preparation techniques are used to concentrate trace amounts of an analyte or alter the sample matrix to make it more amenable to introduction into the analytical instrument. Separation techniques are used to resolve complex mixtures prior to analyte identification and quantification in the detector.

The EPA has developed sampling methods to collect DDT in air using polyurethane foam (PUF), Tenax, XAD-2, and combinations of the three adsorbent media with pumps capable of 1-5 L/min sampling rates (6; 18; 19; 40). These methods, developed and validated for sampling intervals of $\geq$ 4 hrs, generate a time-weighted-average (TWA) concentration, limiting the temporal resolution to periods equal to the sampling interval. These longer sampling intervals are necessary to ensure a sufficient mass of active ingredient (AI), e.g., DDT, is collected to overcome the effect of dilution inherent in the solvent extraction method. Solvent extraction is a sample preparation

method used to remove pesticides, including DDT, from the adsorbent sampling media. The efficiency of the extraction process is described by a modification of the distribution constant (Equations 1-1 and 1-2) (56). Semi-volatile compounds collected from the air, like DDT, have a low extraction efficiency which is overcome with dynamic extraction techniques utilizing pressurized or Soxhlet apparatus (18; 19; 40; 49; 50; 54; 58; 67). The 12-18 h multi-step, solvent extraction process produces a solution of concentrated analytes; in the EPA method the final volume of this solution is standardized (10 mL) (18; 19). Modern GC injectors have a maximal capacity limited by the volume of gas generated when the liquid sample is flash volatilized during sample introduction (56; 62). Typically, a 1-2 μL volume of sample is introduced into the analytical instrument for quantitation resulting in a sample dilution of ≥ 1:5000 (17; 19). If the efficiency of the solvent extraction is 100%, only 0.02% of the collected analyte is introduced into the analytical instrument. Investigators have reported Soxhlet extraction efficiencies of ≤ 100% for DDT-related compounds from homogenized fish (68%-71%) (52), soil (82%-94%) (38), and air sampling media (77%-100%) (40; 50) using solvent mixtures optimized for organochlorine pesticides. The use of these dilution based sample preparation techniques may lead to an inability to detect low levels of airborne DDT that may be present in behavior assay systems and may exert a biological effect on mosquitoes.

The traditional methods used to measure DDT concentrations in air are, like many analytical methods for environmental samples, complicated multi-step procedures. During a 2001 survey of chromatographic scientists, over 80% of the respondents reported using at least two sample preparation techniques for each sample (46). Each

additional step in an analytical method introduces a level of uncertainty that can be propagated, resulting in less precise measurements due to sample recoveries < 100% and inefficiencies inherent in each step of a multi-step method (20; 33; 36; 43; 47; 71).

3. PROJECT OBJECTIVES

3.1 Current Knowledge

Standard environmental sampling methods are insufficient to measure airborne AI in mosquito behavior assays due to three primary limitations: 1) collection intervals exceed the 10-60 min experiment periods used in many mosquito behavior assays (13; 26; 27; 60; 61; 63; 72), 2) relatively large volumes of air would be removed from the assay systems that may perturb the chemical, air flow and mosquito behavior, and 3) solvent extraction techniques used to remove compounds of interest from the sample media prior to analysis reduce method sensitivity and increase method complexity.

Thermal desorption (TD) is an alternative to the cumbersome and complex standard methods described above. By eliminating the 12-18 h sample extraction techniques, methods employing TD sample introduction are both simpler and faster. Typically small tubes (89 mm – 115 mm long x 6 mm o.d.) packed with adsorbent are used to collect and concentrate analytes in sampled air. Analytes collected on these tubes are introduced into the analytical instrument without any sample preparation. Desorption of the analyte from the sampling media is not significant at ambient temperature and relatively high desorption temperatures are needed to shift the distribution constant (Equation 1) in favor of the inert carrier gas used for TD (4). The elimination of all sample preparation techniques simplifies the analytical method. Additionally, there is no dilution of the sample as the entire collected sample can be introduced into the analytical

instrument without the use of solvent. The elimination of sample preparation and the resulting dilution allows the collection of smaller volumes of air for analysis. If the efficiency of the TD sample introduction is 100%, only 0.02% of the sample volume needed for traditional methods can be used, assuming equal analytical method performance. This equates to significantly less sample volume required when using the TD method; thus, sample collection intervals of less than four hours could be used to measure airborne concentrations within previously described mosquito behavior assays systems (13; 26; 27; 60; 61; 63; 72).

Robbat *et al*. developed a TD GC-MS method with a sample probe to detect organochlorine pesticides in water and soil (59). The results of that study demonstrated the ability of a TD GC-MS method to detect as little as 0.5 ng DDT and to accurately quantify (±10% of expected) DDT in the presence of high levels of organic pollutants without sample clean-up or preparation. The method was well suited for analysis of organochlorine pesticides in soil and water, but was not developed to analyze samples of air. Following pesticide application to agricultural fields, Clément *et al*. collected samples of air with Tenax-packed tubes that were analyzed by TD GC-MS to determine the concentration of pesticides (alachlor, atrazine, captan, formothion, lindane, and phosalone) (11). This study demonstrated the strengths of TD methodology including the simplification of the sample preparation process and the ability to measure hourly variations in pesticide concentrations following application.

One approach to overcome some of the limitations of current methods is to use smaller sampling devices; employing tubes packed with adsorptive materials such as carboxen and Tenax (7; 11) for the determination of pesticide air concentrations. These

devices have a smaller pump requirement, shorter sampling times are used, and a smaller amount of solvent is required to extract analytes from the adsorbent. The primary limitation to the use of a smaller sampling device is their small sampling capacity compared to the adsorbent pucks and plugs used in traditional methods. Despite the potential limitation, Briand and Clément independently demonstrated the ability of these smaller sampling devices to collect and concentrate pesticides from air. Briand was able to measure pesticides in air samples collected during two hour sampling events with relatively low flow rates (1-40 L/min), resulting in total sample volumes of 120 L - 480 L. Briand employed solvent extraction due to the potential degradation of thermally labile compounds during thermal desorption. Clément and co-workers employed air sampling with small carboxen and Tenax sampling tubes followed by analysis with TD GC-MS. Detectable levels of the target pesticides were reported with poor recovery and degradation of thermally labile compounds using this method.

3.2 Aims

The goals of this project are to develop and validate a TD GC-MS method for the determination of airborne DDT in mosquito behavior assay systems. On the assumption that TD is more efficient than traditional sampling and analysis methods, this method will be used during short (< 1 hr) sampling intervals similar to those used during mosquito behavior assays. Determination of the concentrations of airborne DDT present in these mosquito behavior assay systems will help define the exposure conditions within spaces containing DDT-treated materials. The ability to generate reproducible exposure conditions during repeat experiments will also be evaluated during these studies.

This work will focus on the development and application of a sample collection and TD GC-MS analysis method. In Chapter 2, a published manuscript entitled "Dichlorodiphenyltrichloroethane (DDT) Determination in Air by Thermal Desorption Gas Chromatography-Mass Spectrometry" describes the development and validation of the TD GC-MS method. The method was employed for the study of steady state DDT levels in a micro-chamber system over a range of temperatures (24°C - 33°C). The application of the TD GC-MS to the determination of airborne DDT concentrations in mosquito behavior systems is described in Chapter 3. This chapter, a submitted manuscript entitled "Determining Airborne Concentrations of Spatial Repellent Chemicals in Mosquito Behavior Assay Systems" outlines method and performance details during the evaluation of laboratory and field mosquito behavior systems. Chapter 4 includes discussion on the relevance of the work presented in this thesis and future work. Additionally, a published manuscript entitled "Identifying the effective concentration for spatial repellency of the dengue vector *Aedes aegypti*", discussed in Chapter 4 includes correlation of mosquito behavior to concentrations of airborne DDT measured with the method described in this thesis.

FIGURES

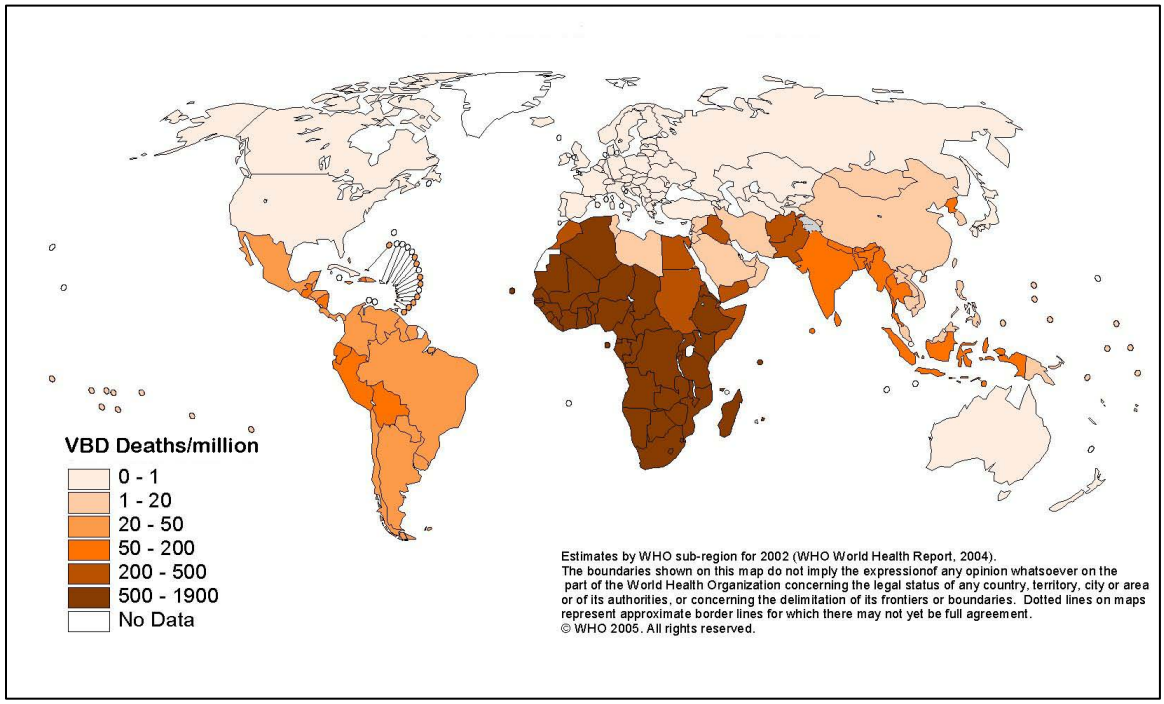

Figure 1-1: Deaths from vector-borne disease. Source World Health Organization http://www.who.int/entity/heli/risks/vectors/en/vbdmap.pdf

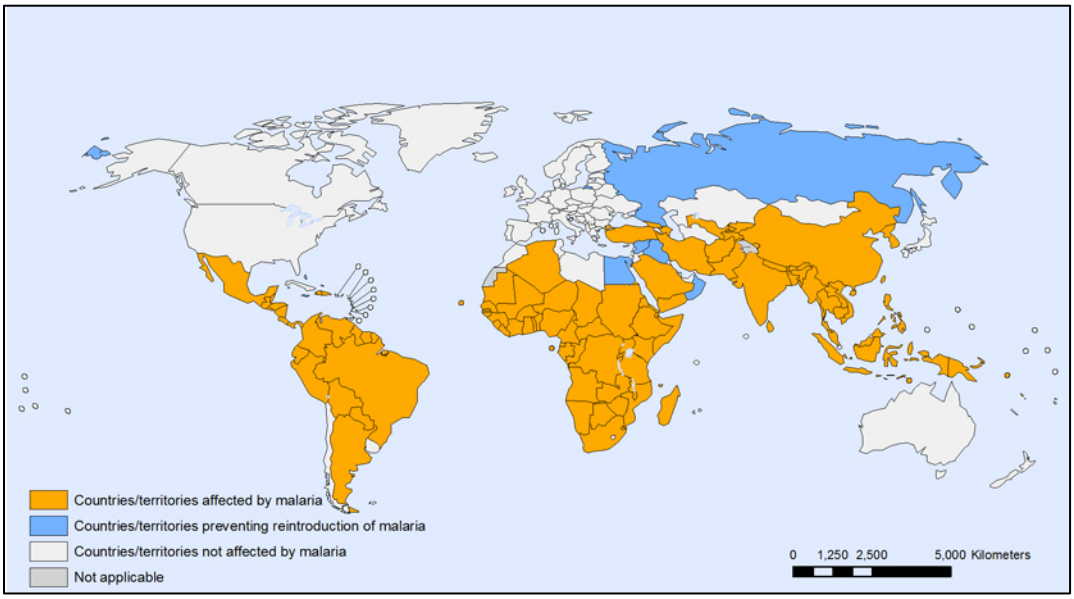

Figure 1-2: Countries and territories affected by malaria, 2010. Source World Health Organization http://gamapserver.who.int/mapLibrary/app/searchResults.aspx

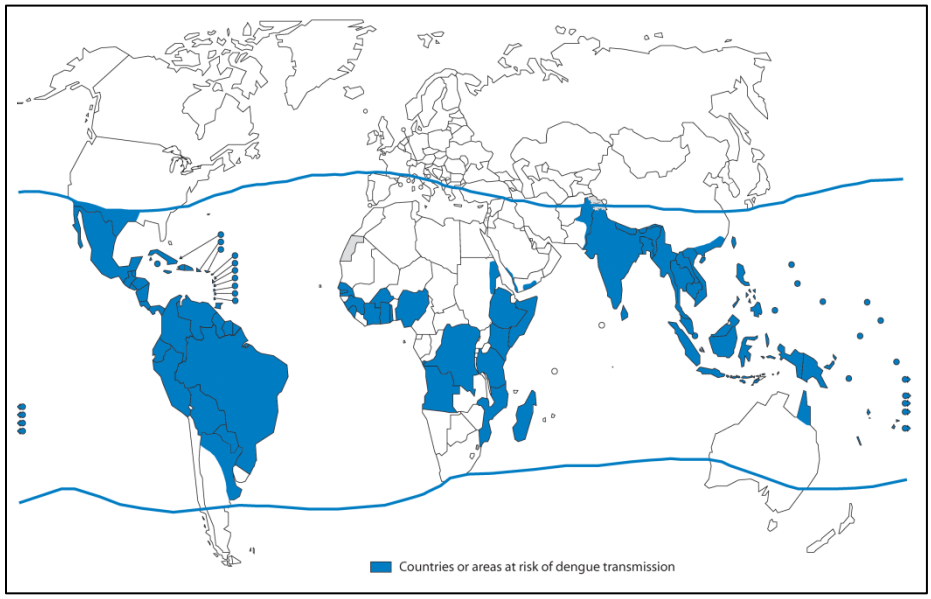

Figure 1-3: Distribution of countries or area at risk of dengue transmission, worldwide, 2008. Source World Health Organization
http://gamapserver.who.int/mapLibrary/app/searchResults.aspx

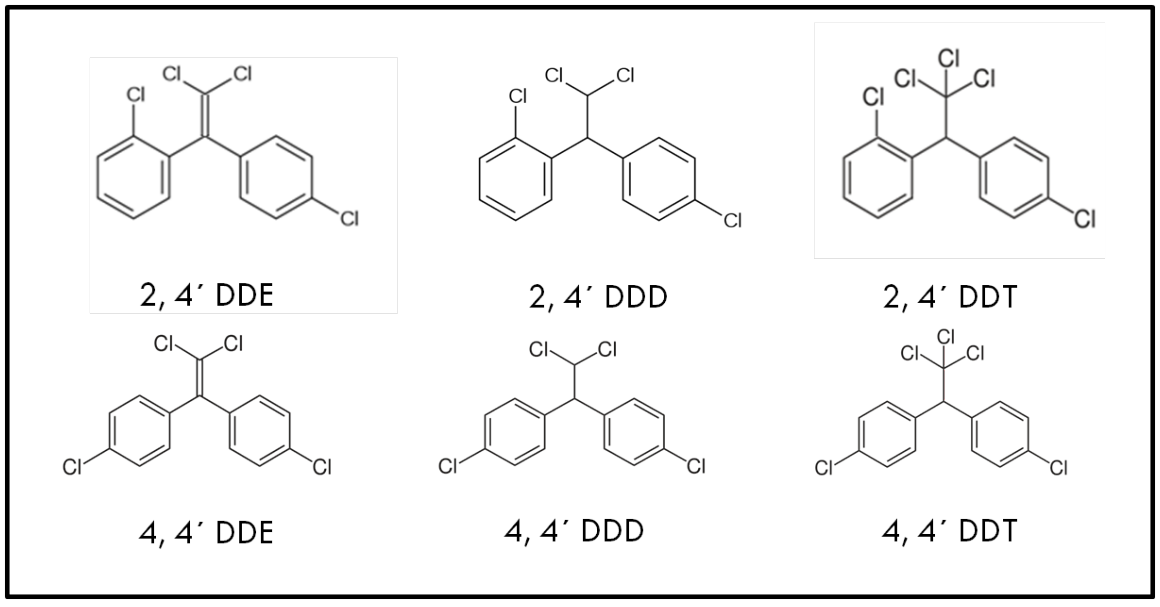

Figure 1-4: Chemical structure of 4, 4´ DDT and related compounds.

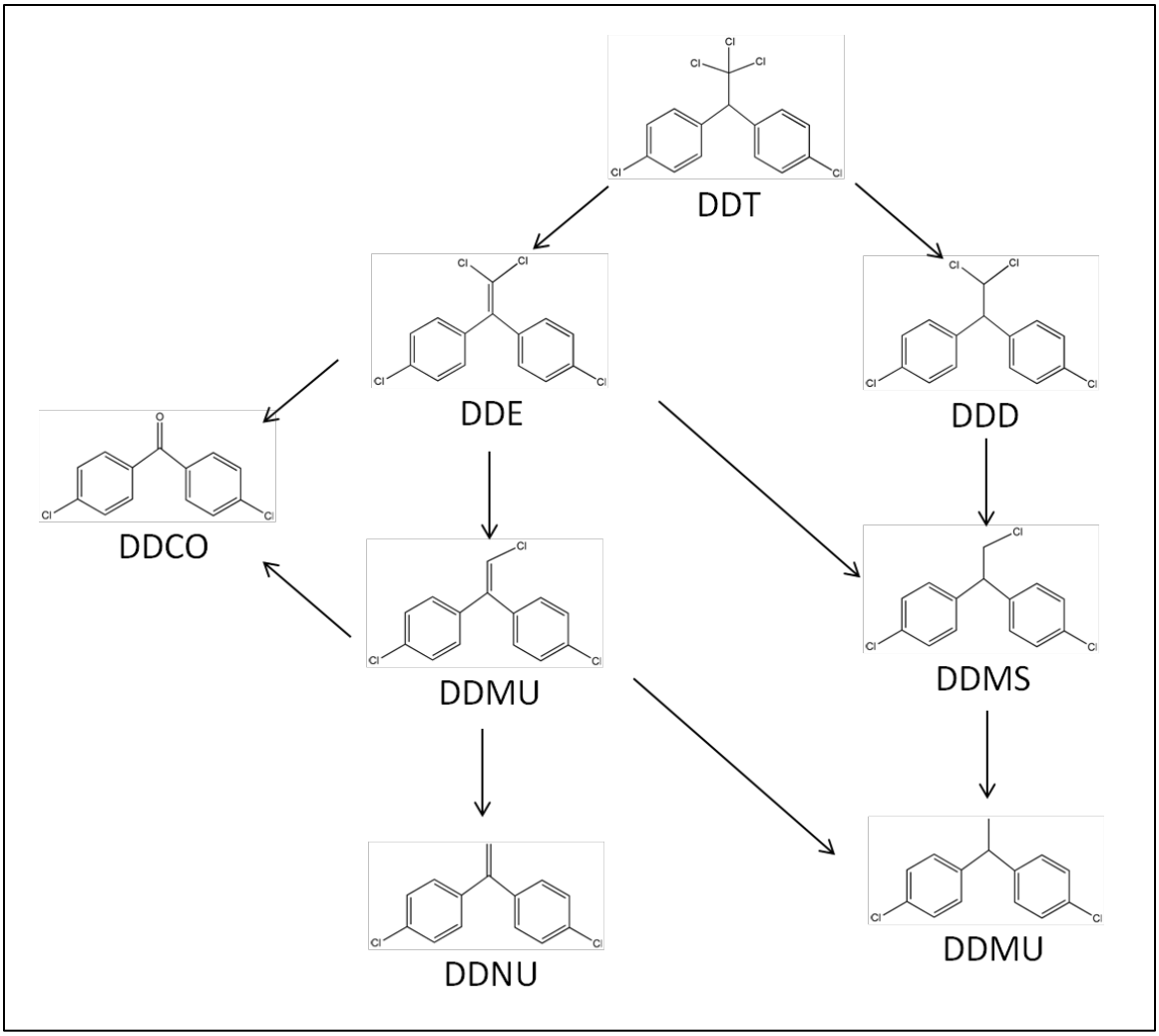

Figure 1-5: Photo aliphatic dechlorination of DDT.

Tables

Table 1-1: Chemicals approved by the WHO for use in IRS and LLINs.

Chemical Class	Chemical Name	Insecticide Action	Indoor Residual Spraying Operations	Long Lasting Insecticide-treated Nets
Carbamate	Bendiocarb	Contact and airborne	X	
	Propoxur	Contact and airborne	X	
Organochlorine	DDT	Contact	X	
Organophsphate	Fenitrothion	Contact and airborne	X	
	Malathion	Contact	X	
	Pirimiphos-methyl	Contact and airborne	X	
Pyrethroid	α-cypermethrin	Contact	X	X
	Bifenthrin	Contact	X	
	Cyfluthrin	Contact	X	X
	Deltamethrin	Contact	X	X
	Etofenprox	Contact	X	X
	λ-cyhalothrin	Contact	X	X
	Permethrin	Contact		X

Table 1-2: Physio-chemical properties of 4, 4′ DDT and related compounds.

	4, 4′ DDT	**2, 4′ DDT**	**4, 4′ DDE**	**2, 4′ DDE**	**4, 4′ DDD**	**2, 4′ DDD**
Synonyms	p, p′ DDT, 1, 1, 1-trichlor-2, 2 bis (*p*-chlorophenyl) ethane,dichloro diphenyltrichlo roethane, DDT	o, p′ DDT, 1, 1, 1-trichlor-2, 2 bis (*o*-chlorophenyl) ethane	p, p′ DDE, 1, 1-dichlor-2, 2 bis (*p*-chlorophenyl) ethylene,dichlo rodiphenyldich loroethylene, DDE	o, p′ DDE, 1, 1-dichlor-2, 2 bis (*o*-chlorophenyl) ethylene	p, p′ DDD, 1, 1-dichlor-2, 2 bis (*p*-chlorophenyl) ethane,dichloro diphenyldichlo roethane, DDD, TDE	o, p′ DDD, 1, 1-dichlor-2, 2 bis (*o*-chlorophenyl) ethane
Chemical Formula	$C_{14}H_9Cl_5$	$C_{14}H_9Cl_5$	$C_{14}H_8Cl_4$	$C_{14}H_8Cl_4$	$C_{14}H_{10}Cl_4$	$C_{14}H_{10}Cl_4$
Molecular Weight	354.49 g/mol	354.49 g/mol	318.03 g/mol	318.03 g/mol	320.05 g/mol	320.05 g/mol
Physical State at Room Temperature	Colorless crystals, white powder	Colorless crystals, white powder	Colorless crystals, white powder	Colorless crystals, white powder	Colorless crystals, white powder	Colorless crystals, white powder
Melting Point	109.0^0C	74.2^0C	89.0^0C	No Data	$109\text{-}110^0$C	$76\text{-}78^0$C
Boiling Point	Decomposes	No Data	336^0C	No Data	350^0C	No Data
Water Solubility	0.025 mg/L at 25^0C	0.085 mg/L at 25^0C	0.12 mg/L at 25^0C	0.14 mg/L at 25^0C	0.09 mg/L at 25^0C	0.1 mg/L at 25^0C
Low K_{ow}	6.91	6.79	6.51	6.00	6.02	5.87
Vapor Pressure	1.6×10^{-7} torr at 20^0C	1.1×10^{-7} torr at 20^0C	6.0×10^{-6} torr at 25^0C	6.2×10^{-6} torr at 25^0C	1.35×10^{-6} torr at 25^0C	1.94×10^{-6} torr at 25^0C

Equations

Equation 1-1: Distribution constant (K_D) at a given temperature, $[A]_E$ is the concentration of analyte in the extracting solvent, and $[A]_M$ is the concentration of analyte in the sampling media.

$$K_D = {[A]_E}/{[A]_M}$$

Equation 1-2: Extraction efficiency (E) equation derived from the distribution constant where V is the ratio between the volume of extracting and sampling media phases ($[A]_E$ and $[A]_M$), and n is the number of extraction cycles.

$$E = 1 - \left[{1}/{(1 + K_D V)^n}\right]$$

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CHAPTER 2: DICHLORODIPHENYLTRICHLOROETHANE (DDT) DETERMINATION IN AIR BY THERMAL DESORPTION GAS CHROMATOGRAPHY-MASS SPECTROMETRY

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ABSTRACT

BACKGROUND: Current quantitative methods for airborne dichlorodiphenyltrichloroethane (DDT) require collection and extraction times of ≥12 h. The aim of this study was to develop a method to quantify airborne DDT with a short (< 4 h) collection and analysis time. RESULTS: Precision (relative standard deviation (RSD) for each calibration point (0.8-9.0), linearity (R^2 = 0.99), and apparent recovery (R′ = 96.5%) were determined from thermal desorption (TD) gas chromatography-mass spectrometry (GC-MS) analyses of Tenax-TA packed sampling tubes spiked with 1 to 250 ng DDT. Recovery of ^{13}C labeled 4, 4′ DDT from tubes spiked before and after air sampling was 97.3% and 90.3%, respectively. DDT was detected and quantified in 1-3 L samples of air collected during 10-180 min sampling events. A significant difference was observed in DDT air concentration between 28 and 33^{0}C during micro-chamber studies. CONCLUSIONS: Our results demonstrate that the TD GC-MS method developed in this study is precise, reproducible, and linear over the span of 1-250 ng DDT spiked onto TD

tubes. By avoiding dilution of the sample, the method described allows the measurement of DDT vapor concentrations during short sampling periods (10-180 min), relevant to mosquito behavior studies.

1. INTRODUCTION

In 2010, the United Nations estimated that indoor residual spraying (IRS) had protected approximately 75 million people at risk to malaria worldwide (27). The World Health Organization has identified 12 pesticides, including Dichlorodiphenyltrichloroethane (DDT; CAS # 50-29-3), for use as part of IRS vector control programs to deter the spread of malaria (25). During IRS operations, insecticide is sprayed onto the interior surfaces of homes and interacts with vectors through mosquito contact with treated surfaces or volatilized chemical.

The analytical methods utilized by the United States Environmental Protection Agency (EPA) for the detection and quantitation of DDT employ solvent extraction of samples collected on adsorbent media (7; 8). Polyurethane foam (PUF), Tenax, or PUF/Tenax adsorbents are commonly used to collect and concentrate DDT from air sampled during 12 h intervals (1; 14). Following solvent extraction, the sample analytes are separated in a gas chromatography (GC) system and detected with a mass spectrometric detector (6; 8). Investigators have applied these methods to determine the concentration of organochlorine pesticides in outdoor (11; 19) and indoor air (20; 23).

The primary limitation of these methods is the reliance on complicated, time-consuming solvent extraction techniques. These techniques can require 12-18 h to remove target analytes from sampling media (7). Additionally, solvent extractions require dilution of the sample which can lead to a loss of analytical sensitivity (>10 µg of analyte must be collected to introduce 1 ng in a typical 1 µl injection into a GC-MS instrument) (10; 13; 17; 18; 24). For samples of air, this effect of dilution can be reduced by collecting large volume samples of air (>1,000 L), but this requires sampling intervals of 12-24 h using sampling pumps capable of 1-5 L/min sampling rates.

Thermal desorption (TD) is an alternative method that does not require solvent extraction steps prior to sample introduction into a GC instrument. The elimination of solvent extraction simplifies the analytical method by reducing the number of sample preparation steps. Additionally, dilution of the sample may be reduced during TD gas chromatography-mass spectrometry (MS) analysis, which allows the collection of smaller volumes of air for analysis. Robbat *et al*. developed a TD GC-MS instrument with a sample probe to detect organochlorine pesticides in water and soil (21). The results of that study demonstrated the ability of a TD GC-MS method to detect as little as 0.5 ng DDT and to accurately quantitate (±10% of expected) DDT in the presence of high levels of organic pollutants without sample clean-up or preparation. The method was well suited for analysis of organochlorine pesticides in soil and water, but was not developed to analyze samples of air. Following pesticide application to agricultural fields, Clément *et al*. collected samples of air with Tenax-packed tubes that were analyzed by TD GC-MS to determine the concentration of pesticides (alachlor, atrazine, captan, formothion, lindane, and phosalone) (4). This study demonstrated the strengths of TD methodology including the simplification of the sample preparation process and the ability to measure hourly variations in pesticide concentrations following application.

The aim of this study was to develop and validate an analytical method to quantify volatilized DDT in air with TD GC-MS analysis with a short collection and analysis time (< 4 h). The TD GC-MS method in this study was developed to collect 1 to 3 L samples of air using metal tubes packed with 200 mg Tenax TA during 10-180 min sampling intervals. Precision, linearity, apparent recovery, DDT degradation, and spiked recovery were examined.

2. EXPERIMENTAL METHODS

2.1 Materials

Analytical standards (Figure 2-1; ≥99% purity) for 2, 4′ and 4, 4′ isomers of dichlorodiphenyldichloroethylene (DDE; Figure 2-1; **1** and **2**), dichlorodiphenyldichloroethane (DDD; Figure 2-1; **3** and **4**), and DDT (Figure 2-1; **5** and **6**) were obtained from Accustandards (New Haven, CT). Ring labeled 4, 4′ DDT (^{13}C at all aromatic carbons) was purchased from Cambridge Isotopes Laboratories (Andover, MA). Stock solutions were prepared in pesticide-free isooctane (Honeywell Burdick and Jackson, Morristown, NJ) and stored at 4 °C in a dark refrigerator. Ultra high purity He and N_2, and 0% relative humidity air (Air Gas, Bethesda, MD) were used for carrier, TD system purge gas, and chamber air supply, respectively. Polyester material (0.023 cm thick white polyester; mesh size 24 x 20 per inch; BioQuip Products Inc, Rancho Dominguez, CA) treated with 4, 4′ DDT was used in all chamber tests.

2.2 Study Design

The primary objective of this study was to develop and validate a TD GC-MS method for the quantitation of airborne DDT. Liquid samples were introduced into the GC-MS instrument without TD to evaluate the ability of the fast GC-MS method used to resolve DDT from related compounds (Figure 2-1). Following development of the GC-MS method, liquid samples were loaded onto TD tubes to evaluate the performance of the TD sample introduction method without air sampling. Samples of air were then collected from a glass chamber during TD GC-MS method development to determine sampling time and flow rate requirements. Precision (relative standard deviation [RSD] of calibration samples), linearity, reproducibility (apparent recovery), DDT degradation,

and spiked recovery were determined to validate the TD GC-MS method. The validated sample collection and analysis method was used to determine the steady state 4, 4´ DDT air concentration in an environmental chamber volatilizing from treated polyester at a range of temperatures (24, 28 and 33 °C).

2.3 Experimental Methods

2.3.1 Sample Introduction

The fast GC-MS method developed by Cajka *et al*. (3) was used for initial analyses of liquid solutions introduced to a standard injection port. Injection volume was 1.0 µl and a split ratio of 5:1 was used with 250 °C injector temperature. Thermal desorption tubes (89 mm X 4 mm i.d. X 6.4 mm o.d.) packed with 200 mg of Tenax-TA adsorbent (Markes International, Llantrisant, UK) were used. Tubes were conditioned at 300 °C for 20 min with a constant N_2 stream (30 mL/min) prior to use to restore tubes to a blank condition.

The TD conditions evaluated were based on methods developed by Clément *et al*. (4) A Unity 2 Thermal Desorber (Markes International, Llantrisant, UK) was connected by a heated transfer line (200 °C) to an Agilent 5975T low thermal mass GC-MS instrument (Santa Clara, CA). The transfer line was connected directly to the GC column to bypass the split-splitless injector. Two flow conditions were tested during method development, a method with split flow and a method without split flow.

A split method was utilized with 75 mL/min flow through the tube during desorption at 300 °C (10 min) onto a focusing trap. The trap was kept at 20 °C during the primary tube desorption with 15 mL/min He flow through the trap, and a 60 mL/min flow to the split vent. The trap was then desorbed onto the GC column without split (1.8

mL/min column flow) at 300 °C (10 min), providing an overall split ratio of 5:1 for this TD method. Dry N_2 purge gas around the cold trap prevented condensation and ice buildup.

The method without split used a 15 mL/min flow through the tube during desorption at 300 °C (10 min) onto the focusing trap. The trap was kept at 20 °C during the primary tube desorption with 15 mL/min He flow through the trap, and no flow to the split vent. The trap was then desorbed onto the GC column without split (1.8 mL/min column flow) at 300 °C (10 min).

Solutions containing a single DDT-related compound (Figure 2-1) were prepared to determine analyte retention times. A 1.0 µl volume of the retention time solution (100 ng analyte in 1 µL isooctane) was quantitatively loaded into a sampling tube. Retention time and elution order were determined for 2, 4′ and 4, 4′ isomers of DDE, DDD and DDT. Laboratory calibration curves were generated by quantitatively loading 1.0 µl of diluted stock solution (1-250 ng 4, 4′ DDT in 1 µL isooctane) onto a TD tube.

2.3.2 GC Separation

A DB-1 (J & W Scientific, Folsom, CA) 30 m × 0.25 mm i.d. × 0.25 µm film thickness fused silica column was used. Separation was completed at constant pressure (12 PSI). The transfer line from the TD system to the resistively heated GC column was passed through the small oven on the 5975T instrument that also housed the split/splitless injector. The transfer line oven and the transfer line to the MS detector were heated to 250 and 280 °C, respectively. Two column temperature programs were used during method development. The initial GC temperature program (column held at 60 °C for 60 s, ramped at 60 °C/min to 300 °C) was used to determine the retention time and elution

order for the 2, 4′ and 4, 4′ isomers of DDD, DDE and DDT (Table 1). To resolve 4, 4′ DDD and 2, 4′ DDT, a two stage GC temperature program based on the work of Clément *et al*. was developed. The GC column temperature was held at 50 °C for 30 s followed by 50 °C/min ramp to 200 °C (no hold), 10 °C /min ramp to 270 °C (no hold), and 30 °C/min to 300 °C (held for 30 s).

A total cycle time of 25 min per sample included a 1 min He purge prior to tube desorption, 10 min primary desorption time, 2 min He purge prior to trap heating, 10 min GC-MS analysis simultaneous with trap heating, and 2 min trap cool down. This allowed the generation of results approximately 25 min following sample collection.

2.3.3 MS Detection

Electron ionization (70 eV) was used with a 2.75 min solvent delay. Selected ion monitoring/scan (SIM/SCAN) mode was used, scanning *m*/*z* 75 to 360 at 3.75 scans/s. Quantitation was performed by calculating the area under the curve (AUC) for *m*/*z* 165 and 235 SIM data with the GC-MS data handling system (Chemstation version E.02.00.493; Agilent Technologies).

2.4 Airborne DDT Sample Generation and Collection

Samples of airborne DDT were generated by placing polyester fabric treated with 4, 4′ DDT in a temperature controlled chamber. A DDT solution (18 mg/mL isooctane) was applied using a micropipette method described by Said *et al.* (22) to achieve 0.2 mg DDT loading per cm^2 of fabric.

A glass chamber (5.4 cm diameter x 6.0 cm height; Kimble Chase, Vineland, NJ) was utilized for method development. Treated polyester (180 cm^2) was placed against

the inner wall of the chamber. The chamber was placed in a heater block (Digi Block; Barnstead/Thermo Scientific, Dubuque, IA) to control temperature. The transfer lines (R3606 Tygon Tubing; Saint-Gobain Performance Plastics, Aurora, OH) and TD tube were at ambient temperature (24-26 °C). Air flow into the glass chamber and through the TD tube from the air supply tank was controlled with a needle valve and measured with a solid state flow meter (Model 6000; Restek, Bellefonte, PA).

Studies on DDT volatization rate and the recovery of labeled DDT spiked to sorbent tubes were performed in a Micro-chamber/Thermal Extractor (μ-CTE; Markes International). Air flow was controlled by the μ-CTE pressure regulator and verified with a volumetric flow rate meter (Defender 510, Bios International, Butler, NJ). The temperature during DDT steady state studies was 24, 28, or 33 °C (±1 °C), and 33 °C (±1 °C) to study the recovery of labeled DDT spiked to sorbent tubes.

2.5 ^{13}C DDT Spiked Recovery

To determine the recovery of labeled 4, 4′ DDT, 100 ng ^{13}C ring labeled DDT dissolved in 1.0 μL nonane was loaded onto the metal screen in front of the Tenax adsorbent within a TD tube. Samples of air (2 L) were collected simultaneously from the μ-CTE chambers containing polyester treated with unlabeled 4, 4′ DDT during 20 min intervals. Four sampling conditions (n = 6 for each test condition) were evaluated: 1) no labeled 4, 4′ DDT loaded onto sampling before or after air sampling collection, 2) labeled 4, 4′ DDT loaded onto sampling tubes without collection of air, 3) labeled 4, 4′ DDT loaded onto sampling tubes after collection of air, and 4) labeled 4, 4′ DDT loaded onto sampling tubes before collection of air. Retention time and extracted ion chromatograms

(EIC) were used to identify labeled (*m/z* 247) and unlabeled (*m/z* 235) DDT, and related compounds.

2.6 Statistical Analysis

Statistical analyses were completed in Sigma Plot for Windows (Version 11.0, Systat Software, Chicago, IL). Specific statistical methods are described for each data set. A p value of less than 0.05 was considered to indicate statistical significance.

3. Results and Discussion

3.1 GC Separation

During analysis of liquid (Figure 2-2A) and spiked TD sampling tubes (Figure 2-2B) with the initial GC temperature program (60 ˚C/min), 4, 4´ DDE, DDD, and DDT were detected (GC peaks 2, 4, and 6, respectively). Additional DDT-related compounds were detected during analysis of samples of air collected from the glass chamber system that contained 4, 4´ DDT-treated polyester (Figure 2-3A). Several of these compounds were identified as 2, 4´ isomers of DDT and degradation compounds (GC peaks 3 and 5, respectively, Figure 2-3A). Similar to the findings described by Cajka *et al*. (3), complete baseline resolution of 4, 4´ DDD (GC peak 4, Figure 2-3A) and 2, 4´ DDT (GC peak 5, Figure 2-3A) was not obtained using the initial fast GC temperature program, complicating the identification of DDT-related compound concentrations in samples of air.

Complete baseline resolution was not obtained with the two-stage GC temperature program (Figure 2-3B), but the time between the maxima of the 4, 4´ DDD and 2, 4´ DDT peaks was 0.12 min compared to 0.05 min with the 60 °C/min ramp. This GC temperature program was not optimized for the separation of 4, 4´ DDD and 2, 4´ DDT;

additional method development would be required to resolve and quantify these compounds. Despite this limitation, 4, 4′ DDD and 2, 4′ DDT can be identified in a sample containing both compounds with this two-stage GC temperature program. The two-stage GC temperature program was used for subsequent analyses.

3.2 TD Sample Introduction Conditions

Analyses of TD sample tubes spiked with 100 ng 4, 4′ DDT using either split or splitless conditions were performed to evaluate 4, 4′ DDT recovery and degradation. The median GC-MS detector response (AUC) during split and splitless TD was 5.75×10^7 and 3.75×10^8 area counts, respectively; the distribution of the two groups differed significantly (Mann-Whitney Rank Sum Test; $U = 0$; $p < 0.001$). Based on the split ratio measured, approximately five times the DDT present in the sampling tube is introduced to the GC-MS during splitless TD resulting in greater MS detector response. The mean baseline was less than 10,000 for ($n = 10$) split and greater than 12,500 for splitless ($n = 10$) samples. Detector response was still quantifiable from baseline for injections of 1 ng DDT using the split method which translated to a quantification limit of 0.08 and 1.0 $\mu g/m^3$ corresponding to 6 and 69 ppt for 12 and 1 L samples of air, respectively. The percent DDT degradation observed during split and splitless TD was not significantly different (Mann-Whitney Rank Sum; $U = 38$; $p = 0.860$).

The median peak width included 30 and 24 scans/peak for split ($n = 9$) and splitless ($n = 10$) TD samples, respectively. The difference in median peak width between the two TD methods was statistically significant (Mann-Whitney Rank Sum Test; $U = 0.0$; $p < 0.001$), but did not impact peak identification or integration of 4, 4′ DDT by the GC-MS data handling program. The split TD method was used for

subsequent analysis based on the similar DDT degradation pattern observed during split and splitless TD, reduced mean baseline values, and sensitivity in the ppt range.

3.3 Method Validation

3.3.1 Precision and Linearity

Liquid injections into the split/splitless injector produced a linear GC-MS response ($R^2 = 0.990$) with analysis of 1 to 250 ng DDT. The RSD for each calibration point ranged between 4.5 and 30.1. The split TD method analyses for DDT spiked to a TD tube produced a linear GC-MS response ($R^2 = 0.991$) over a range of 1 to 250 ng DDT. The RSD for each calibration point ranged from 0.8-9.0. The GC-MS response was different between the two sample introduction methods when comparing the same DDT mass which may be due to differences in the precision of split control during liquid injections (electronic pressure control) and TD (manual split control).

3.3.2 Apparent Recovery

The apparent recovery (R´) was calculated to evaluate the bias (over or under-estimation) and reproducibility of the method per Burns *et al*. (2). The R´ value was determined by repeat analysis of low (25 ng; n = 10), medium (50 ng; n = 10), and high mass (100 ng; n = 5) DDT samples. The R´ values ($R'_{25\ ng} = 100.7\%$; $R'_{50\ ng} = 93.4\%$; $R'_{100\ ng} = 95.5\%$) were biased toward under-estimating the volatile DDT air concentration ≥50 ng. However, the R´ values were within the range (±15%) accepted by the EPA (8).

3.3.3 DDT Degradation

Degradation of DDT was observed at all mass loading levels following both liquid injection and thermal desorption (Figure 2-4). The percent DDT degradation was

assessed by dividing the sum of the peak areas for DDD and DDE by the sum of the peak areas for DDD, DDE and DDT (8). The mean DDT degradation observed for all masses from liquid injection was 1.68% (n = 22; SD = 0.75%). A one-way analysis of variance (ANOVA) showed a significant difference between the percent degradation observed during the analysis of liquid samples with 1-250 ng DDT (overall $F = 72.679$; $p < 0.001$). Post-hoc tests using the Holm-Sidak method showed that percent DDT degradation with liquid injection varied significantly between low mass samples (1, 5 and 10 ng DDT) and high mass samples (50, 100, and 250 ng DDT). Increased degradation (as a relative percent) at lower mass loading did not noticeably affect the GC peak area linearity for DDT analyses using liquid sample introduction.

The mean DDT degradation following TD was 25.66% (n = 38; SD = 19.59%). The data were not distributed normally (Shapiro-Wilk Normality Test; $p < 0.05$); a Kruskal-Wallis one-way ANOVA on ranks showed a significant difference in the percent degradation observed during the analysis of TD samples with 1-250 ng DDT (overall $H = 19.337$; $p = 0.004$). Post-hoc tests using Tukey's method showed that relative percent degradation with TD analysis varied significantly between low spiked samples (1, 5 and 10 ng DDT) and high spiked samples (50, 100, and 250 ng DTT). Increased percent degradation at lower mass loading did not noticeably affect overall linearity for DDT analyses using TD for sample introduction.

Measured percent degradation of DDT was significantly different between the liquid and TD methods (t-test; $p < 0.001$). For TD analyses the relative percent degradation decreased with the analysis of higher DDT mass values in an exponential manner ($R^2 > 0.999$) suggesting a saturable degradation process.

Degradation products observed during analyses of spiked sample tubes were consistent over the entire mass range tested, with 4, 4′ DDD and 4, 4′ DDE identified based on comparison to retention times and mass spectra of analytical standards. Three additional compounds, 2, 4′ DDD, DDE and DDT were detected during analysis of samples of air from chambers containing polyester fabric treated with 4, 4′ DDT. The formation of 2, 4′ and 4, 4′ isomers of DDD and DDE from 4, 4′ DDT exposed to light has been described(5; 9; 15) and for 4, 4′ DDT in water (12).

3.3.4 Spiked Recovery

2, 4′ DDT was detected in samples of air collected from the glass chamber containing polyester treated with 4, 4′ DDT. To determine if 2, 4′ DDT formed during the sample collection or analysis process, labeled 4, 4′ DDT was loaded onto TD tubes either before, after, or without the collection of samples of air from the μ-CTE. Extracted ion chromatograms were used to identity labeled (*m/z* 247; Figure 2-5 right column) and unlabeled (*m/z* 235; Figure 2-5 left column) 4, 4′ DDT in each sample. The unidentified peak in tubes spiked with ^{13}C DDT (denoted with asterisk; Figure 2-5 D, F, and H) is likely labeled 4, 4′ DDD (analogous to GC peak 4 in Figs. 5A, 5E, and 5G) based on retention time. However, a ring labeled standard for 4, 4′ DDD was not used during this experiment to confirm the identity of this peak. The labeled 4, 4′ DDT GC-MS response (AUC) for samples spiked before, after, and without the collection of 2 L air was not significantly different between groups (Kruskal-Wallis one-way ANOVA on ranks; H = 2.351; p = 0.309). Spiked recovery exceeded 90% for samples spiked before (97.3%) and after (90.3%) the collection of air.

The 2, 4′ DDT detected in samples of air (Figs. 3A, 3B, 5E, and 5G) may be formed in the sampling chamber. Differences in response factor for 2, 4′ and 4, 4′ DDT were compared during analysis of TD samples spiked with solutions containing equal amounts (1-100 ng) of both analytes. The response factor for 2, 4′ DDT was consistently higher, with a mean response factor of 1.19 for TD samples (SD = 0.10; range: 1.03-1.37). The response factor difference and potential for 2, 4′ DDT (≤ 2%) to be present in the 4, 4′ DDT solution used to treat polyester materials does not fully account for the 2, 4′ DDT detected in samples of air. The formation of 2, 4′ DDT does not appear to be an artifact of sample preparation or the TD GC-MS method.

3.4 Chamber Studies

The 4, 4′ DDT air concentration in the μ-CTE was determined by simultaneously collecting 180 min samples of air from the six stainless steel chambers. The mean air sampling rate was 12.82 mL/min (SD = 0.24 min; range 12.2-13.7 mL/min). Steady state (no statistical difference between DDT air concentrations measured at different time points) was achieved after 27 h (Figure 2-6).

The median steady state DDT air concentration was 5.6, 6.1, and 12.8 $\mu g/m^3$ for the 24, 28, and 33 °C chamber studies, respectively. The data were not distributed normally (Shapiro-Wilk Normality Test; $p < 0.05$); a Kruskal-Wallis one-way ANOVA on ranks showed a significant difference between groups (overall $H = 62.000$; $p < 0.001$). Post-hoc tests using Tukey's method showed that median DDT air concentration measured during the 33 °C chamber study was significantly higher than the median DDT air concentrations measured during the 24 and 28 °C chamber studies ($p < 0.05$ for both comparisons). Vapor pressure is an important parameter for predicting a compound's

volatility (16). The significant increase in steady state 4, 4′ DDT air concentration observed between 28 and 33 °C is similar to the increase in vapor pressure reported over a similar temperature range (26).

4. CONCLUSIONS

Our results demonstrate that the TD GC-MS method developed in this study was precise, reproducible, and linear over the span of 1-250 ng DDT. By eliminating the dilution of the sample associated with solvent extraction >90% of the collected 4, 4′ DDT may be introduced into the GC-MS instrument. The primary limitation of this method is the degradation of DDT during TD GC-MS analysis which complicated the ability to measure DDT degradation that may have occurred in the environmental chamber prior to sample collection. Additional work is planned to further optimize this TD GC-MS method for the analysis of airborne DDT to identify and reduce, if possible, the cause of DDT degradation during analysis. The detection of 2, 4′ DDT in samples of air was not expected and may have implications for studies on the effects of 4, 4′ DDT on mosquito behavior.

The variations in the DDT air concentrations observed during 75 h studies in the μ-CTE would not have been detected with traditional sample techniques that rely on sampling periods >3 h with the collection of >100 L of air. The short sampling time intervals associated with this method allowed the measurement of fluctuations in the DDT air concentration related to temperature and time since chemical application. The results from our study demonstrate the ability to measure DDT vapor concentrations during 10-180 min intervals. This method can be used to quantify airborne DDT in laboratory or field test systems employed in mosquito behavior studies. These studies

should focus on the impact of environmental factors on the DDT generation rate and how this rate is related to mosquito behavior and the life-span of treated materials. Based on the results obtained, the development of TD GC-MS methods to study short-term volatilization of other semi-volatile insecticides and repellants is planned.

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The views expressed in this article are those of the author and do not necessarily reflect the official policy or position of the Department of the Navy, Department of Defense, nor the U.S. government. The primary author is a military service member, and this work was prepared as part of his official duties. Title 17 U.S.C. §105 provides that 'Copyright protection under this title is not available for any work of the United States Government.' Title 17 U.S.C. §101 defines a U.S. Government work as work prepared by a military service member or employee of the U.S. Government as part of that person's official duties.

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FIGURES

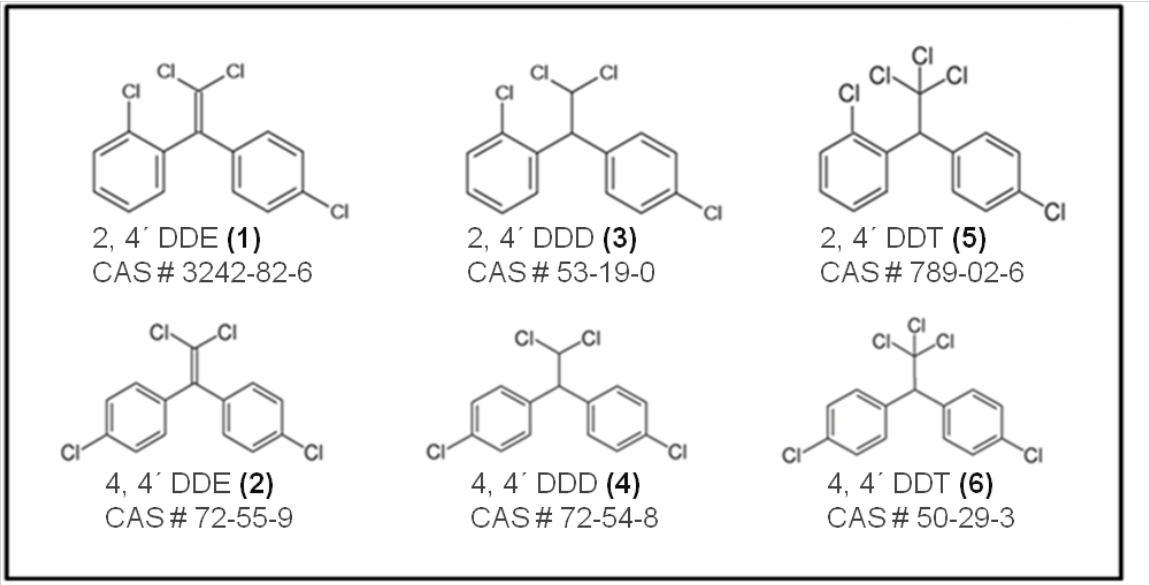

Figure 2-1: Structures of DDT and related compounds.

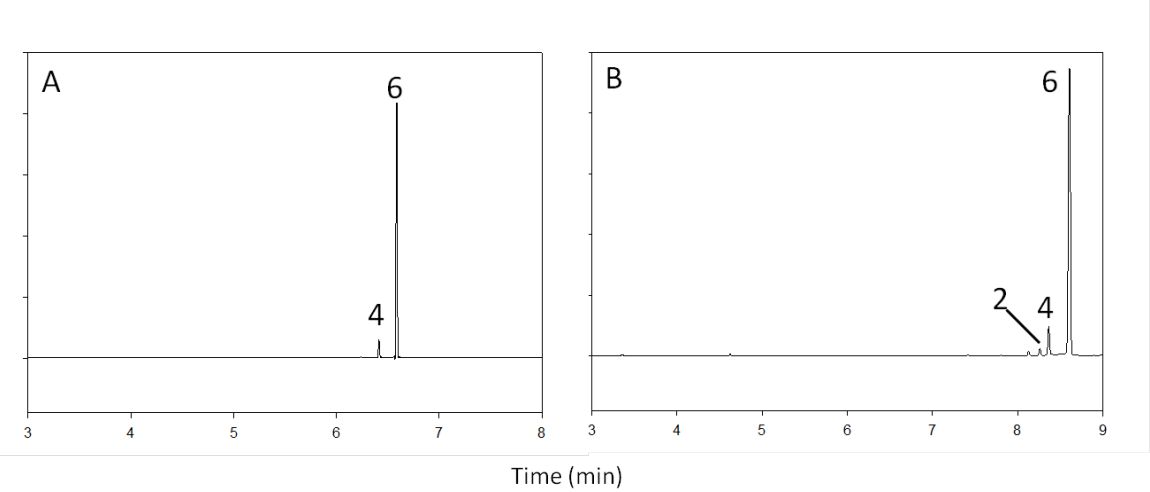

Figure 2-2: Selected ion (*m/z* 235, 165, and 246) chromatograms of DDT and related compounds observed following injection of a 100 ng liquid standard (A), and TD of a tube spiked with 100 ng DDT liquid standard. Separation was performed with the initial temperature program. The initial temperature (60 °C) was held for an additional 1.5 min during TD analysis resulting in a retention time shift when comparing the liquid injection (A) and TD tube analysis (B). GC peak labels are identified in Table 1.

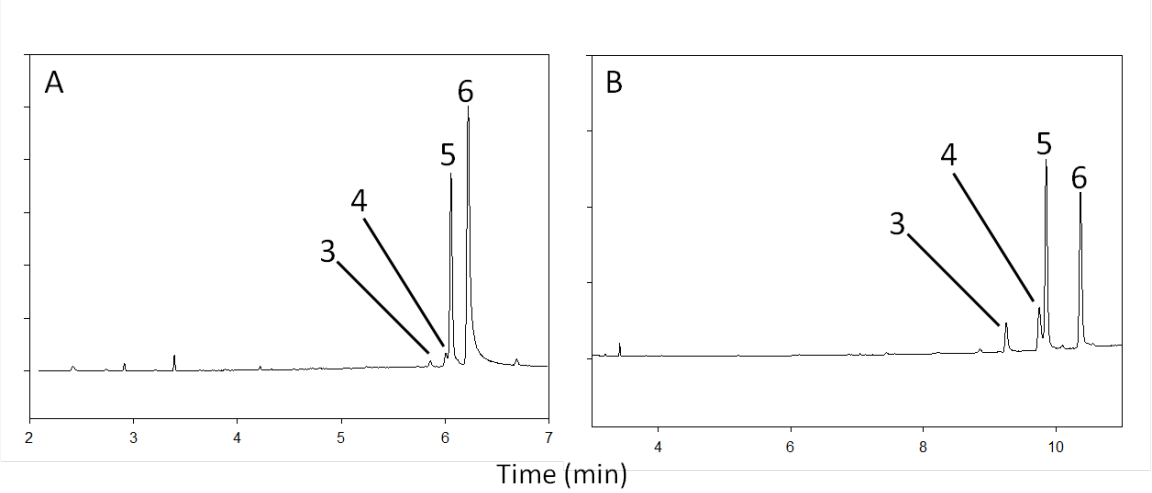

Figure 2-3: Selected ion (*m/z* 235, 165) chromatograms of DDT and related compounds observed with TD GC-MS analysis of air samples collected on a tube from a glass chamber containing fabric treated with 98% 4, 4′ DDT in isooctane. Separation was performed with the initial (A) or two-stage (B) GC temperature program. GC peak labels are identified in Table 1.

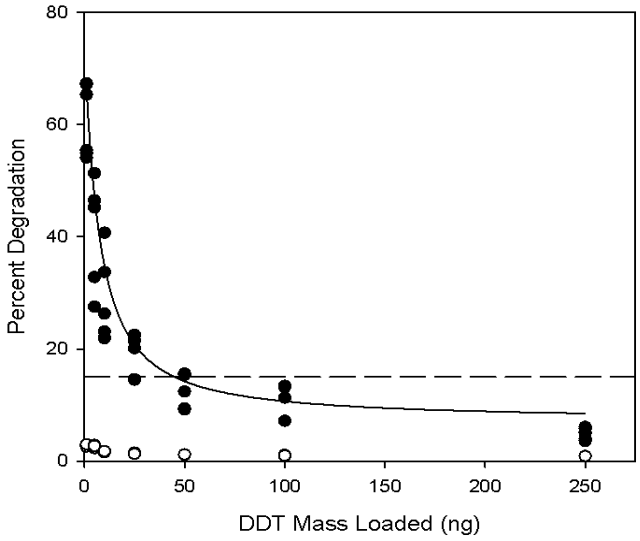

Figure 2-4: DDT degradation observed during liquid injection (open circles) and TD sample introduction (solid circles). The dashed line identifies 15% DDT degradation specified as acceptable per EPA Method 8081B.

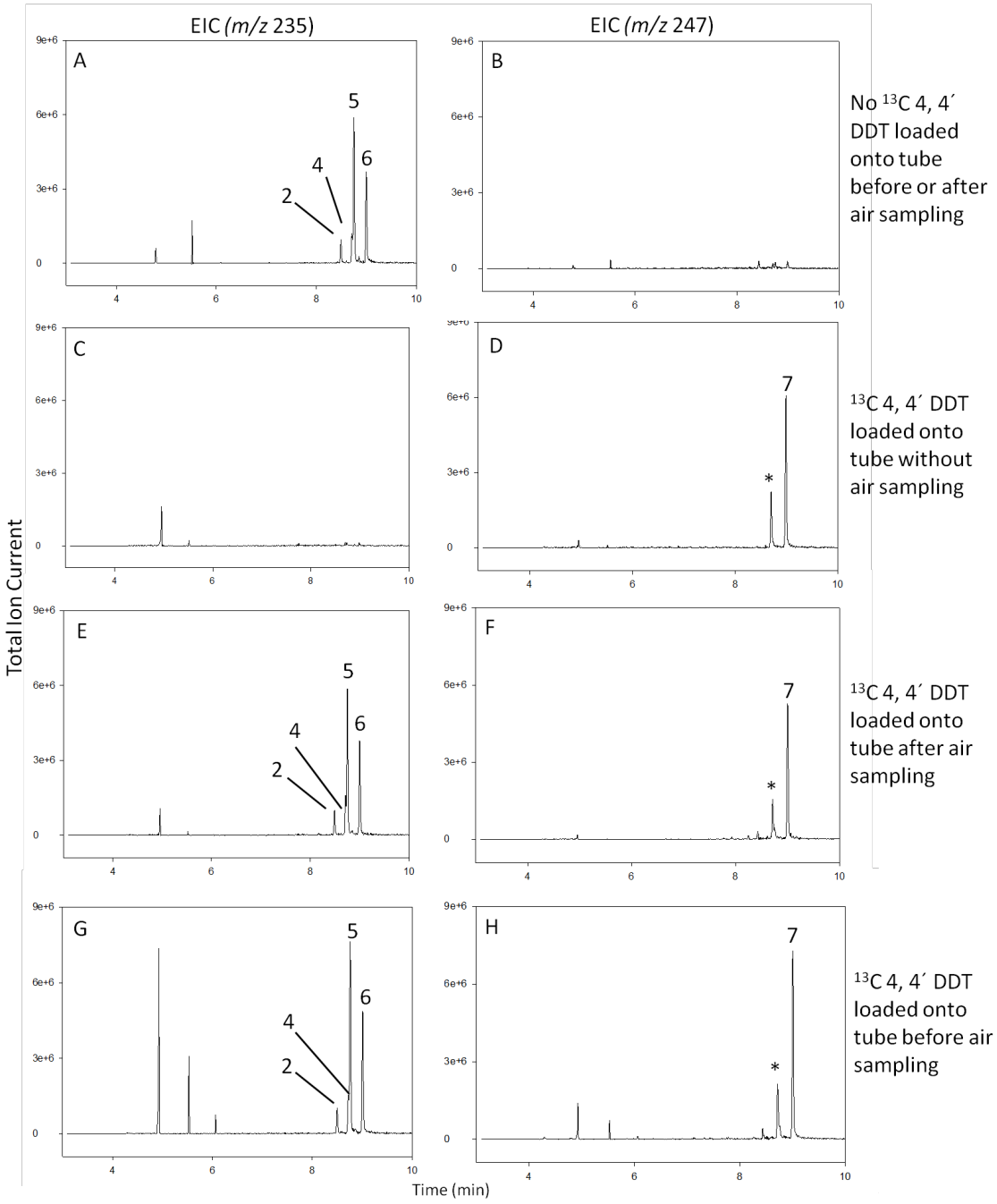

Figure 2-5: Extracted ion chromatograms demonstrating the presence of analytes related to unlabeled DDT (*m/z* 235, left column), and ring-labeled 4,4' DDT (*m/z* 247, right column). Sampling conditions used to evaluate ^{13}C ring labeled 4, 4´ DDT recovery are listed to the right of the chromatograms. Each row includes *m/z* 235 and 247 chromatograms extracted from the same sample. GC peak labels are identified in Table 1, the unidentified peak in tubes spiked with ^{13}C DDT (denoted with asterisk in D, F, and H) is likely labeled 4, 4´ DDD.

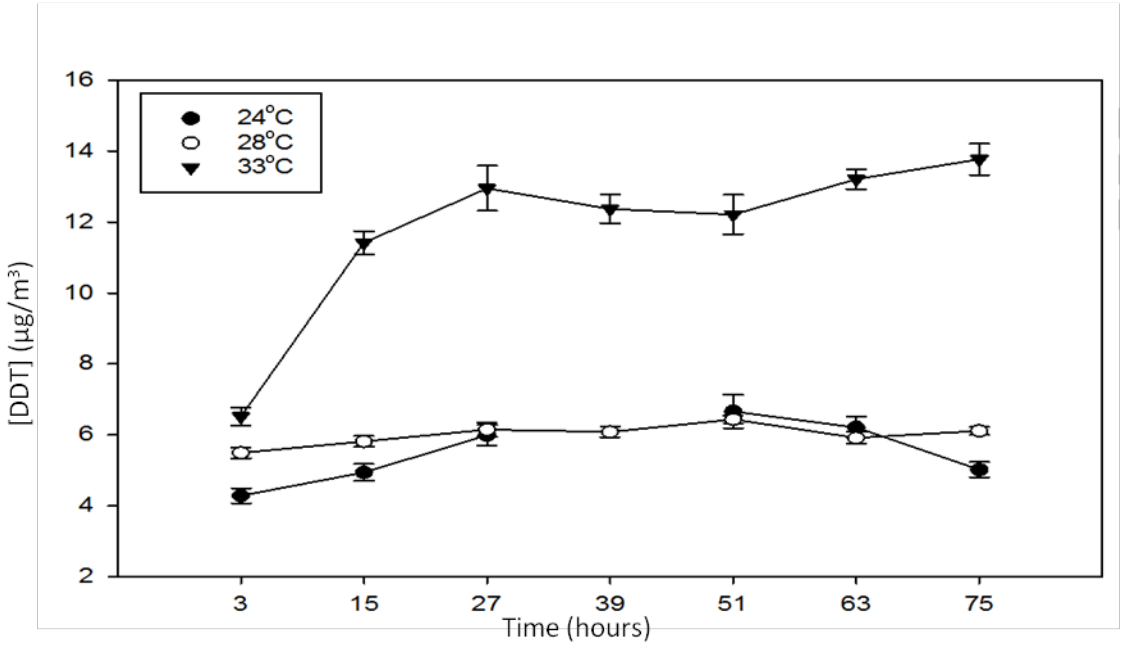

Figure 2-6: DDT air concentration determined during µ-CTE experiments at 24 (solid circles), 28 (open circles), and 33 °C (solid triangles). The median steady state DDT air concentration measured at the various temperatures was significantly different (Kruskal-Wallis one-way ANOVA on ranks; $p < 0.001$). Post hoc tests using Dunn's method showed that median DDT air concentration measured during the 33 °C chamber study was significantly higher than the median DDT air concentrations measured during the 24 and 28 °C chamber studies.

TABLES

Table 2-1: Retention time and characteristic ions of DDT and related compounds analyzed with fast GC-MS method.

Chemical	M.W. (u)	Mean RT (min)[a]	Selected Ions (*m/z*)	GC Peak Label (Figs. 2, 3, and 5)
2, 4′ DDE	316	7.94	246 $[M\text{-}Cl_2]^+$	1
4, 4′ DDE	316	8.08	246 $[M\text{-}Cl_2]^+$	2
2, 4′ DDD	318	8.14	235 $[M\text{-}CHCl_2]^+$ 165 $[M\text{-}CHCl_2\text{-}Cl_2]^+$	3
4, 4′ DDD	318	8.31	235 $[M\text{-}CHCl_2]^+$ 165 $[M\text{-}CHCl_2\text{-}Cl_2]^+$	4
2, 4′ DDT	352	8.35	235 $[M\text{-}CHCl_3]^+$ 165 $[M\text{-}CHCl_3\text{-}Cl_2]^+$	5
4, 4′ DDT	354	8.54	235 $[M\text{-}CHCl_3]^+$ 165 $[M\text{-}CHCl_3\text{-}Cl_2]^+$	6
13C 4, 4’ DDT	366	9.01[b]	247 $[M\text{-}CHCl_3]^+$	7

a. n=3

b. ^{13}C DDT sample analysis completed using a different DB-1 GC column. Labeled and unlabeled 4, 4′ DDT did not have significantly different retention times, labeled DDT RT = 9.011 min, unlabelled DDT RT = 9.013 min (t-test; p = 0.432).

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CHAPTER 3: DETERMINING AIRBORNE CONCENTRATIONS OF SPATIAL REPELLENT CHEMICALS IN MOSQUITO BEHAVIOR ASSAY SYSTEMS

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Abstract

Background: Mosquito behavior assays have been used to evaluate the efficacy of vector control interventions to include spatial repellents (SR). Current analytical methods are not optimized to determine short duration concentrations of SR active ingredients (AI) in air spaces during entomological evaluations. The aim of this study was to expand on our previous research to further validate a novel air sampling method to detect and quantitate airborne concentrations of a SR under laboratory and field conditions. **Methodology/Principal Findings:** A thermal desorption (TD) gas chromatography-mass spectrometry (GC-MS) method was used to determine the amount of dichlorodiphenyltrichloroethane (DDT) in samples of air. During laboratory experiments, 1 L volumes of air were collected over 10 min intervals from a three-chamber mosquito behavior assay system. Significantly higher levels of airborne DDT were measured in the chamber containing textiles treated with DDT compared to chambers free of AI. In the field, 57 samples of air were collected from experimental huts with and without DDT for onsite analysis. Airborne DDT was detected in samples collected from treated huts. The mean DDT air concentrations in these two huts over a period of four days with variable ambient

temperature were 0.74 μg/m^3 (n = 17; SD = 0.45) and 1.42 μg/m^3 (n = 30; SD = 0.96).

Conclusions/Significance: The results from laboratory experiments confirmed that significantly different DDT exposure conditions existed in the three-chamber system establishing a chemical gradient to evaluate mosquito deterrency. The TD GC-MS method addresses a need to measure short-term (< 1 h) SR concentrations in small volume (<100 L) samples of air and should be considered for standard evaluation of airborne AI levels in mosquito behavior assay systems. Future studies include the use of TD GC-MS to measure other semi-volatile vector control compounds.

Key Words: thermal desorption (TD), gas chromatography (GC), mass spectrometry (MS), dichlorodiphenyltrichloroethane (DDT), field portable, near real-time, spatial repellency (SR), indoor residual spraying (IRS), mosquito behavior assays

1. INTRODUCTION

Mosquitoes are capable of transmitting numerous diseases including malaria, dengue fever, yellow fever, Japanese encephalitis, and West Nile fever among others (11; 19). Due to the geographic distribution of mosquitoes, as many as three billion people are at risk of infection with at least one mosquito-borne disease (21; 24). Of those at risk, malaria causes the highest burden of disease with an estimated 216 million cases and 655,000 deaths reported in 2012 (24). In addition, infection with one of the four serotypes of dengue virus is responsible for up to 400 million infections annually (4), with up to 500,000 cases progressing to the life-threatening dengue hemorrhagic fever (21).

Two of the primary strategies to control mosquito-borne diseases as recommended by the World Health Organization (WHO) are the use of long-lasting insecticide-treated nets (LLINs) and indoor residual spraying (IRS) to reduce exposure to mosquitoes (20; 23). However, only twelve compounds in four chemical classes are currently available for LLINs and IRS (20). In an effort to identify new active ingredients (AI) and/or innovative chemical paradigms of vector control, such as the use of spatial repellents (SR) to modify mosquito behavior (1; 2), entomological assays have been developed to describe specific vector response following exposure to an AI (6; 10; 25). These include both laboratory and field test systems that measure repellency (deterrence or reduction in mosquito entry), irritancy (increased exit), and mortality (5-7; 14; 15). Dichlorodiphenyltrichloroethane (DDT), a compound approved by the WHO for use in IRS operations, has been the focus of anopheline behavioral evaluations. In subsequent studies, SR activity of DDT has also been evaluated against both male and female *A. aegypti*

mosquitoes (16; 18). Combined, these studies demonstrate that DDT elicits SR activity in mosquito vectors (22).

At the time the studies mentioned previously were conducted, there were no published analytical methods to measure the concentration of airborne DDT over short sampling intervals (≤ 1.0 h); therefore, the concentration of DDT relevant to SR activity in test systems could not be determined with temporal resolution. Although defining the short-duration concentration of airborne DDT was not a specific objective of previous evaluations, it is now recognized as a critical component in the development of novel or reformulated vector control compounds. This is because an understanding of the specific conditions required to generate sufficient airborne concentrations of a SR chemical to repel mosquitoes will allow identification of operationally significant parameters relevant to SR control strategies. These parameters include product format, placement in a given space (e.g., home), required AI loading levels to elicit minimum thresholds of mosquito responses, effective distance, and environmental conditions such as temperature, humidity, and wind speed, that may affect airborne SR concentrations.

Here we report on a thermal desorption (TD) gas chromatography-mass spectrometry (GC-MS) method, previously developed in our laboratory (13), to determine the concentrations of airborne DDT in samples of air collected from laboratory and field mosquito behavior assay systems. Specific objectives included: 1) validating a difference in airborne DDT concentrations from spaces with and without DDT treatment and 2) describing the role of the TD GC-MS method to measure concentrations of airborne AI.

2. EXPERIMENTAL METHODS

2.1 Ethics Statement

Permission was obtained from the Thailand Armed Forces Development Command prior to conducting field evaluation in Pu Teuy Village, Sai Yok District, Kachanaburi Province, Thailand (14u209110N, 98u599450E).

2.2 Materials

Analytical standards (≥ 99% purity) for 2, 4′ and 4, 4′ isomers of dichlorodiphenyldichloroethane (DDD), dichlorodiphenyldichloroethylene (DDE), and DDT were obtained from Accustandards (New Haven, CT). Stock solutions were prepared in pesticide-free isooctane (Honeywell Burdick and Jackson, Morristown, NJ) for laboratory experiments or reagent grade acetone (Fischer Scientific, Pittsburgh, PA) for field experiments. Stock solutions were stored in the dark at 4˚C until testing. Ultra high purity (UHP) He and N_2, acquired from local suppliers, were used for carrier gas and TD system cold trap dry purge gas respectively, during laboratory (Air Gas, Bethesda, MD) and field (Air Gas, Bangkok, Thailand) experiments.

2.3 Analytical Methods

A TD GC-MS method, previously developed in our lab (13), was used for near real-time analysis of laboratory and field samples. For field analyses, the TD GC-MS instrument and supporting equipment items were shipped to Bangkok, Thailand and later transported to the experimental hut site of Pu Tuey Village. The TD GC-MS instrument was operational within 24 h of transportation to the field site.

2.3.1 Sample Introduction

A Unity 2 thermal desorber (Markes International, Ilantrisant, UK) was connected by a heated transfer line (200°C) to an Agilent 5975T GC-MS instrument (Santa Clara, CA) with a low thermal mass (LTM) column assembly. The transfer line was connected directly to the analytical column through the heated injector body with the liner removed.

Laboratory calibration curves were generated by quantitatively loading 1.0 µl of diluted stock solution (1.0-250.0 ng DDT in 1.0 µL isooctane) into a sampling tube. Control samples (sample tubes spiked with known amounts of DDT) were analyzed every 10-20 samples. Experimental samples were not analyzed if controls were not within ±15% of expected values. Standards of DDT in isooctane were prepared in Bethesda, MD and were packaged according to international shipping requirements for transport at ambient temperature to Thailand for field calibration of the TD GC-MS system.

A two-stage split TD method was used with 75 mL/min flow through the tube during desorption at 300°C (10 min) onto a low volume focusing trap. The trap was maintained at 20°C during primary tube desorption with 15 mL/min He flow through the trap and a 60 mL/min flow to the split vent. The trap was then ballistically heated to 300°C and the focused analytes were transferred onto the GC column without split (10 min), providing an overall split ratio of 5:1 for this TD method.

2.3.2 GC-MS Analysis

A DB-1 open tubular fused silica analytical column was used (J & W Scientific, Folsom, CA; 30 m × 0.25 mm i.d. × 0.25 µm film thickness), with helium used as the desorption and carrier gas for separation completed at constant pressure (12 PSI). The transfer lines from the heated

injector body to the resistively heated LTM GC column and from the small convection oven to the MS detector were maintained at 250°C and 280°C, respectively. The initial GC column temperature was held at 50°C for 30 s, followed by a 50°C/min ramp to 200°C (no hold), 10°C/min ramp to 270°C (no hold), and 30°C/min to 300°C (held for 30 s).

Electron ionization (70 eV) was used with a 2.75 min solvent delay. Selected ion monitoring (SIM)/scan mode was used, scanning *m*/*z* 75 to 360 at 3.75 scans/s, providing at least 10 scans across the relevant GC peaks. Quantitation was performed using *m*/*z* 165 and 235 SIM data.

2.4 Sample Collection

Samples of air were collected using tubes (89 mm × 4 mm i.d. × 6.4 mm o.d.) packed with 200 mg of Tenax-TA adsorbent (Markes International Ilantrisant, UK). Tubes were conditioned at 300°C for 20 min with a constant N_2 stream (30 mL/min) prior to use. Low-flow personal air sampling pumps (Model 222, SKC Inc., Eighty Four, PA) were set to operate with a flow rate of 100 or 200 mL/min in the laboratory and field, respectively. Pumps were calibrated before and after sampling using a device to measure volumetric flow rate (Defender 510, Bios International, Butler, NJ).

2.4.1 Time-delayed Analysis

During field experiments, samples were analyzed following variable delays post-collection. To assess the impact of time-delayed analysis on DDT recovery, replicate TD tube samples (n = 4) were prepared by spiking 100 ng DDT in 1.0 µl isooctane onto the metal screen

of a TD tube at the sampling inlet. Analysis by TD GC-MS was conducted immediately, and after delays of one or three days. Before analysis, sampling tubes were sealed with brass caps and polytetrafluoroethylene ferrules and stored at 4°C.

2.4.2 Laboratory Sample Collection

A three-chamber mosquito behavior assay system was used for laboratory evaluations (Fig 1) (12). The three chambers represented: treatment (containing DDT-treated textile); central (point of mosquito introduction); and control (containing DDT-free fabric). Each chamber was a 28.4 L cube with a 10 cm hole cut into a removable clear acrylic lid. The treatment and control chambers were constructed from metal with acrylic lids and were fitted with beveled funnels that allow passage of mosquitoes originating from the central chamber during tests. The central chamber was made entirely of clear acrylic.

Chemical treatment was matched to standard mosquito behavior evaluation protocols. White polyester (mesh size 24 x 20/inch; Bioquip Products, Rancho Dominquez, CA) or nylon (No 4-2; G Street Fabrics, Rockville, MD) textile was treated with DDT solution at 0.09 – 2.0 g/m^2, corresponding to 0.4-100% of the WHO recommended IRS loading rate (23) using acetone and isooctane diluents as described previously (16). Textile was prepared to cover 100%, 75%, 50% or 25% surface area of treatment and control chambers. Control fabric was prepared with solvent only. Fabric panels were treated 30-60 min prior to starting an assay and allowed to air-dry on a drying rack for 15-30 min before placement in the test system. The material remained in the treatment or control chambers for the duration of a test day.

Filtered air (5.0 L/min measured with a rotameter (RMA-5-BV Flowmeter, Dwyer Instruments, Inc., Michigan City, IN) for each inlet was supplied to the assay system through two inlets; one each in the treatment and control chambers. The 10 L/min of supplied air was exhausted from the system through the mosquito introduction chamber. Before air sampling was performed each chamber was filled with argon and a hand-held thermal conductivity detector was passed along the surfaces of the chamber joints to determine if the welded and sealed joints were airtight.

Airflow velocity was measured at 27 points within each chamber to determine if differences in airflow existed within and between the chambers using an anemometer (VelociCalc 9555, Thermo Scientific Inc., Shoreview, MN). Air changes per hour in the treatment chamber were determined by introducing a high concentration of CO_2 into the chamber and then measuring the decay of this gas with a portable meter equipped with a non-dispersive infrared absorbance detector (MultiRAE IR, RAE Systems, San Jose, CA) (3).

The sampling pumps were kept outside of the test chambers during sample collection and connected to the sample tubes by inert tubing (R3606 tubing; Saint-Gobain Performance Plastics, Aurora, OH). Pump flow rate (100 mL/min) was checked daily, both before and after sample collection with a sampling tube inline, to verify sampling rate was within ± 5% of the set value. The average pump flow rate and sample collection time were used to calculate the volume of air sampled. The temperature and relative humidity of the testing room (recorded at the start of each day) were 26°C-31°C and 10%-20%, respectively.

2.4.3 Field Sample Collection

Air samples were collected from inside experimental huts used for mosquito behavior evaluations (17). The construction and design of the experimental huts has been previously described (6). Briefly, huts were 4.0 m wide, 5.0 m deep and 2.5 m tall, with three windows (1.1 m x 1.2 m) and one door (0.8 m x 2.0 m) comprising a total internal volume of 50 m^3. Chemical treatment matched laboratory evaluations. Polyester fabric (19.8 m^2 total per hut) that corresponded to 50% of the interior wall surface area was treated with 2.0 g/m^2 of DDT dissolved in acetone one day prior to placement in treatment huts (huts B and C). Polyester textile treated with acetone only was positioned inside the control hut (hut A).

In the field, samples of air were collected during 60 min intervals inside the three experimental huts (Fig 2). The flow rate of the sampling pumps was measured through a representative sample collection tube at 200 mL/min (± 2 mL/min) before and after sample collection. The average pump flow rate and sample collection time were used to calculate the volume of air sampled. Pumps were mounted on wooden stands in the center of each hut approximately 1.5 m above the floor of the hut. Samples were collected over 1 h intervals between 0600-1800 during 9-12 October 2010. Outdoor temperature and wind speed were measured outdoors at a location central to the huts while the temperature was measured continuously inside each hut. The indoor air change rate was determined using the decay of CO_2 by the same portable gas meter used to make similar determinations in the three-chamber laboratory system.

2.5 Statistical Analysis

Statistical analyses were completed in Sigma Plot for Windows (Version 11.0, Systat Software, Chicago, IL). Analysis of variance (ANOVA) was used to assess the impact of delayed analysis on the mass of DDT remaining in spiked sampling tubes immediately, and after delays of one and three days. For laboratory evaluations, the inter-day and inter-chamber variations in the concentration of airborne DDT were evaluated by ANOVA, comparing the concentrations measured in the treatment on different testing days and in different chambers, respectively. Differences in the airflow rate measured in each chamber of the laboratory mosquito behavior assay were compared by ANOVA. Holmes-Sidak (parametric) and Tukey (non-parametric) *post-hoc* tests were performed for all analyses (as appropriate). A p value of less than 0.05 indicated statistical significance for all analyses.

3. RESULTS

3.1 Laboratory Sampling

Argon leakage was detected at each non-welded seal indicating the box model system was not airtight and that air could be supplied or removed from the system independent of the inlets and exhaust. The median air velocity was 1.0 cm/s in each chamber of the box model system. A Kruskal-Wallace one-way ANOVA test did not demonstrate a significant difference between chambers ($H = 1.104$; $p = 0.576$). Higher air velocities (2.5-21.8 cm/s) were measured directly below the inlets in the treatment (Fig 1B) and control (Fig 1D) chambers.

A total of nine samples of air (1.0 L) were collected during 10 min sampling periods over a three day period to assess the stability of airborne DDT concentrations in the chamber containing DDT treatment (100% coverage at 0.09 g/m^2) (Fig 1B). The intra-day variation was assessed by calculating the daily relative standard deviation (RSD). The RSD was 16.3%,

13.8%, and 7.0% for days 1-3, respectively. Inter-day variance was assessed to determine the effect of sample preparation (polyester independently prepared each day before placement in the test chamber) and time (Fig 3). Results showed a significant difference between the mean DDT air concentrations measured on each of the three days ($F = 33.664$; $p < 0.001$). The DDT air concentration measured on Day 3 was significantly higher than levels measured on Days 1 and 2 (Holm-Sidak *post hoc*; $p < 0.001$).

Examination of chamber-specific DDT air concentrations using 25% coverage at 2 g/m^2 indicated large intra-chamber variation (>100%; n = 9). The intra-chamber variation calculated for each chamber was 110%, 139% and 197% for treatment, central and control, respectively. This may be due in part to the percentage of samples below the limits of quantitation (treatment: 11.1%, central: 37.0%, and control: 18.5%) and detection (treatment: 7.4%, central: 55.6%, and control: 63.0%). Samples between the limit of quantitation and detection were assigned the value of half the limit of quantitation (0.5 ng) and samples without detectable levels of DDT were assigned the value 0 ng.

The median concentration of airborne DDT was not significantly different in the treatment chamber between the three days (Kruskal-Wallis ANOVA; $H = 5.190$; $p = 0.075$) indicating that a similar concentration of airborne DDT was generated during the three-day experiment. However, the median concentration of airborne DDT was significantly different between the three chambers (Fig. 4; Kruskal-Wallis ANOVA; $H = 35.461$; $p < 0.001$) with median concentration significantly higher in the treatment chamber compared to the central and control chambers (Tukey *post hoc*; $p < 0.05$).

3.2 Field Sampling

The results of field analyses are summarized in Table 1. The TD method produced a linear GC-MS response ($R^2 = 0.933$) from TD tubes spiked in the field with DDT (5.0 to 100.0 ng). Relative standard deviations were 49.7, 26.0, 18.8, 15.1 and 24.3 for the 5 ng, 10 ng, 20 ng, 50 ng and 100 ng calibration points, respectively. Method performance in the field did not match that performance obtained in the laboratory with respect to linearity, precision, and sensitivity (13). To account for this, the $\text{variance}_{\text{DDT Predicted}}$ was calculated for 10 and 50 ng loading values (9). The mean $\text{variance}_{\text{DDT Predicted}}$ was ± 4.895 ng (10 ng: ± 4.38 ng; 50 ng: ± 5.41 ng) resulting in a $\text{variance}_{\text{DDT Predicted}}$ for calculated DDT air concentrations of ± 0.41 μg DDT/m^3 air. A total cycle time of 25 min per sample allowed a sample throughput of approximately two samples per hour in the field. This relatively short analysis time (compared to ~18h with conventional solvent extraction) facilitated completion of near real-time DDT detection and quantitation. Analyses of the control (n = 18) and hut (n = 57) samples were completed in approximately 40 h.

Fifty-seven samples of air were collected with TD tubes from the three experimental huts (Fig 2). Overall, the amount of airborne DDT measured in samples of air collected during the four days at the field site ranged from non-detectable to 4.30 μg/m^3 (Table 1). DDT detection occurred in 83% of samples from treated huts (huts B and C) and in one sample from the control hut (labeling error suspected) as previously reported (1). While quantitation of airborne DDT concentration was completed by measuring the area under the curve for SIM analysis of 4, 4′ DDT (Fig 5B; peak 4), three other DDT-related GC peaks were also noted. The earlier eluting peaks are likely DDT degradation products 2, 4′ DDD (Fig 5B peak 1), 4, 4′ DDD (Fig 5B peak 2), and the DDT isomer 2, 4′ DDT (Fig 5B peak 3) based on elution order and corresponding full scan mass spectra (13). The mean indoor air temperature measured in each hut (Table 1) did not

show a statistically significant difference between huts. The air change rate measured in hut C was approximately six changes per hour (~300,000 L/hr) based on tracer gas decay measurement, with replacement air supplied by the three windows, one door, and through the walls (determined by visual smoke test). The air volume collected during each sampling interval (~12 L) represented ~ 0.004% of the total volume present in the field system.

Time-delay analysis experiments indicated the mean recovery from sampling tubes spiked with DDT was 94.4 ng (n = 4; SD = 6.6 ng), 89.6 ng (n = 4; SD = 3.3 ng), and 86.1 ng (n = 4; SD = 6.1 ng) for samples analyzed immediately, after one day (mean delay 23.12 h), and after three days (mean delay 72.96 h), respectively. A one-way ANOVA did not demonstrate a significant difference between groups ($F = 2.279$; $p = 0.158$). Additionally, the DDT recovery following one and three days delayed analysis were acceptable (±15% of the starting DDT mass) as defined by the U.S. Environmental Protection Agency (EPA) for analysis of control samples (8). This suggests that delays of up to three days between sample collection and analysis did not impact DDT recovery from TD sampling tubes.

4. DISCUSSION

Quantifying the concentrations of airborne SR chemicals during laboratory and field mosquito behavior studies is critical to understanding the relationship between chemical exposure and mosquito behavior [26]. Such information can be used, in part, to establish entomological correlates of health outcomes related to human protection such as percent reduction in mosquito entry into a treated space, or biting rates. This report describes important performance details for a TD GC-MS analytical method introduced previously [26], to quantify concentrations of airborne DDT in both laboratory and field mosquito assay systems.

Standard environmental sampling methods were not designed to measure airborne AI in samples collected during 10-60 min intervals used in the mosquito behavior assays evaluated in this report. Additionally, these methods rely on solvent extraction to remove compounds of interest from the sample media prior to analysis, reducing the method sensitivity and increasing the analytical method complexity. The TD GC-MS method developed previously [20] and described in detail in this report addresses the limitations of the standard methods with respect to sampling duration with a simplified sample introduction method. All sample preparation was eliminated with the TD GC-MS method as metal tubes packed with sampling media were inserted directly into the TD unit following sampling collection reducing method complexity and analysis time. Sample recovery was also improved compared to traditional methods; we previously reported > 90% sample recovery [20] compared to < 1% possible with solvent extraction. The TD GC-MS method was sensitive enough to measure airborne DDT samples of air collected during 10 and 60 min intervals collected from the mosquito behavior systems in the laboratory and field, respectively. Collection of large volumes of air from mosquito behavior systems could have unintended impact on the behavior of AI and mosquitoes within the system. In the field system approximately 0.004% of the total air volume was sampled during each sampling interval, reducing the impact on the system dynamics with respect to air change rate, AI emission rate and chemical movement.

Longer duration samples provide information regarding the time-weighted average airborne AI concentrations, but cannot provide temporal resolution of high and low concentration values that occur throughout the sampling interval. Efforts to understand mosquito behavior following exposure to SR must include measurement of airborne AI concentrations

over brief periods of time to ensure that excursions above and below an average concentration can be identified and correlated with altered insect behavior. The use of sorbent sampling with TD-based analyte introduction provides a substantial improvement for sampling a dynamic field system in which AI concentrations are expected to fluctuate due to uncontrolled environmental conditions. A short-duration TD method is also important for measuring AI concentration fluctuations in laboratory systems assumed to be stable.

During the assessment of the laboratory assay system, significantly higher concentrations of airborne DDT were observed within the treatment chamber compared to the central and control chambers. This finding validated the assumption that mosquitoes placed in the introduction chamber (central chamber) would be exposed to airborne DDT and that a gradient exists between the treatment (highest concentration) and the control (lowest concentration) chambers. This finding supports reports on the SR action of DDT by other investigators in which mosquitoes exposed to DDT-treated materials, but not in direct contact with the material, were repelled from entering the treated space [17,18,27].

As measured by daily RSD, the intra-day variation of airborne DDT in the three-chamber system (< 20%) indicate that the replicates are similar and acceptable under EPA testing criteria [25,28]. Significant differences were observed under laboratory conditions in the concentration of airborne DDT in the treatment chamber measured on different days using treated material newly prepared for each experiment. This difference suggests the amount of DDT that becomes airborne varies by day, although volume and concentration of DDT solution used for material treatment are held constant, which could affect the repeatability of mosquito behavior studies evaluating the same (nominal) treatment conditions. The differences observed are not likely to

be a result of sampling or analysis method performance as spiked control samples were within 15% of expected values for laboratory experiments. However, the variation in DDT levels may be due to fluctuations in ambient temperature of the testing room (26-31°C) as it has been shown that the steady state air concentration [20] and vapor pressure [29] of DDT increase in a non-linear fashion at temperatures greater than 28°C. Additional explanations for the inconsistent air concentration of DDT in the treatment chamber include: potential degradation of DDT stock solutions prior to fabric treatment, variations in delivery rate or consistency in the fabric treatment procedure, use of a system that was not air-tight, and sampling under non-equilibrium conditions. However, until correlations can be made regarding thresholds for behavioral responses in mosquito test populations and AI airborne concentrations, the true effect of this variability is unknown. Future studies are planned to investigate the impact of each of these potential confounders on the concentration of airborne DDT within the laboratory.

Although our earlier reports have described air sampling outputs in conjunction with deterrent (SR) mosquito responses [26], this is the first detailed description, to our knowledge, of the conditions and performance of a method for near real-time detection of DDT in samples of air collected under field conditions. The on-site method appeared to be sufficiently sensitive to detect levels well below those that would be acutely toxic to humans (1.0 mg/m3) [30] with a quantitation limit of 0.461 μg/m3 (27 ppt) DDT during field sampling and analyses. Additionally, the sample collection method developed for this study was relatively simple allowing on-site training of technicians for sample collection. The results of the field analyses indicate DDT was present in the treatment huts and not in the control hut, confirming mosquitoes approaching or entering treatment huts would be exposed to airborne DDT. While the samples

collected during this study did not cover each hour of the four-day test period, it could be possible to use the 1 h sampling period to collect consecutive samples to measure variations in the concentration of airborne DDT over time.

A limitation of the method employed for on-site sample analysis was the differences in the method performance, with respect to linearity and intra-sample variability of the controls. These differences may be a result of the operating conditions encountered at the field site (23-32°C; relative humidity 65-100%) compared to those in the laboratory (24-27°C; relative humidity 40-60%). Additionally, strict control of the calibration solutions used during field analysis was not possible, as temperature-controlled shipping options were not used.

The primary strength of this study is the evidence provided that airborne DDT was generated in the laboratory and field test systems used to evaluate mosquito vector behaviors. The data support the conclusion that mosquitoes placed in these systems will be exposed to DDT without landing on treated surfaces. Potential confounders such as material treatment and temperature were identified during these experiments, and these should be controlled or accounted for during future air sampling evaluations. More importantly, the sampling and analysis methods described here validate the role of TD GC-MS in entomological evaluations and overall utility in SR product development. Near real-time analysis can identify operational conditions to optimize for maximum SR product effects. Evaluation of the suitability of TD GC-MS methods for sampling other spatial repellent compounds, such as semi-volatile pyrethroids, as well as other chemical classes that are typically used for vector control is warranted.

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The views expressed in this article are those of the author and do not necessarily reflect the official policy or position of the Department of the Navy, the Department of Defense or the U.S. government. The primary author is a military service member, and this work was prepared as part of his official duties. Title 17 U.S.C. § 105 provides that 'Copyright protection under this title is not available for any work of the United States Government'. Title 17 U.S.C. § 101 defines a U.S. Government work as work prepared by a military service member or employee of the U.S. Government as part of that person's official duties. The authors would like to thank Dr. Theeraphap Chareonviriyaphap for coordinating with the Thailand Armed Forces Development Command, Kanchanaburi Province to acquire necessary approvals for conducting studies at the Thailand field site. We also thank Suppaluck Polsomboon and Joko Hendarto for their contribution to sampling activities.

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FIGURES

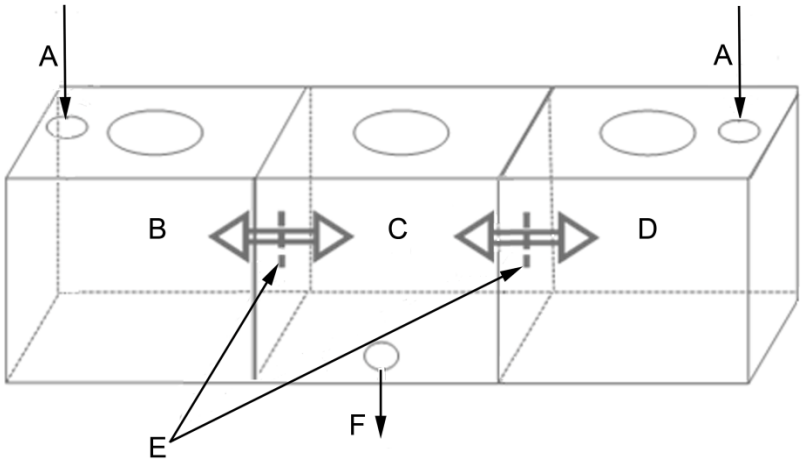

Figure 3-1: A schematic diagram of the three chamber system used to study mosquito behavior. Each chamber was 30.5 cm x 30.5 cm x 30.5 cm (28.4 L) with a 10 cm hole cut into a removable acrylic lid. A: lab air supply (5 L/min) measured with a rotameter, B: metal treatment chamber, C: acrylic mosquito introduction chamber, D: metal control chamber, E: closable funnels opened during exposures studies to allow mosquitoes, air flow, and airborne chemical to move between the chambers, F: vacuum exhaust (10 L/min).

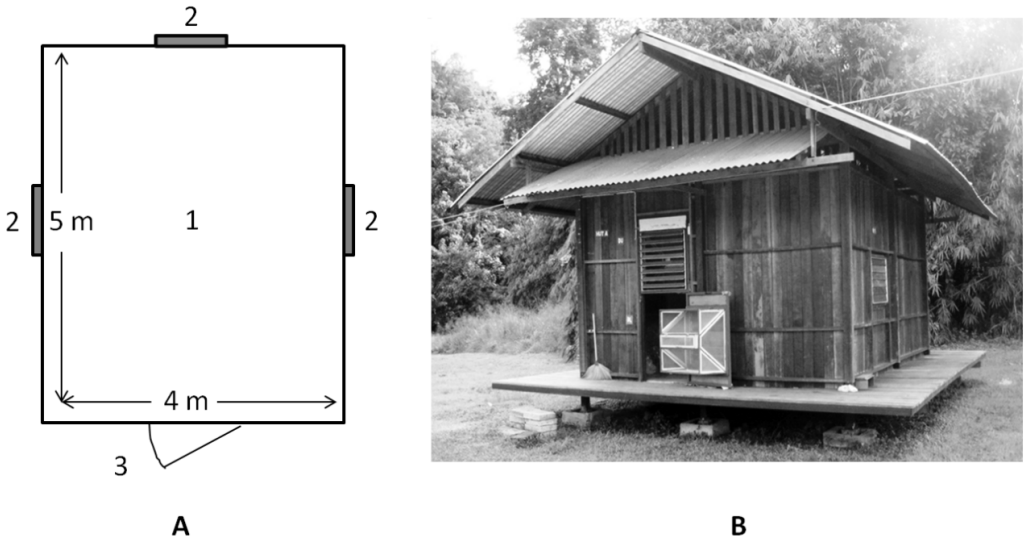

Figure 3-2: Diagram (A) and picture (B) of experimental huts. The sampling pumps were placed on 1.5 m tall stands in the approximate center of each hut (#1 panel A). Each hut had three screened windows (#2 panel A) and one screened door (#3 panel A) allowing air into the hut from the outside.

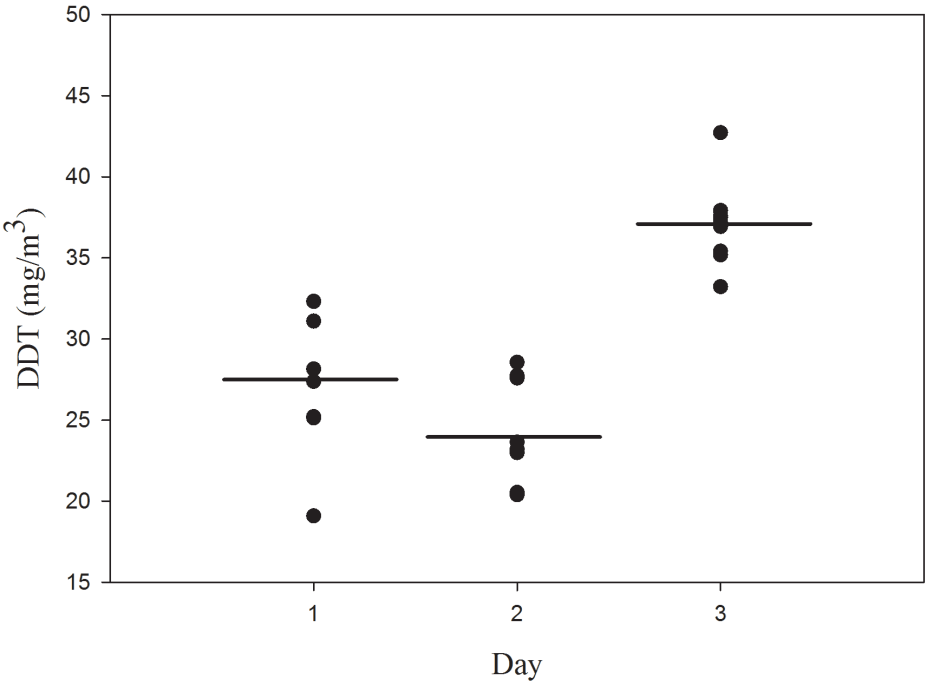

Figure 3-3: Scatter plot of DDT air concentration in samples collected on three separate days from the treatment chamber of the three chamber system. Polyester fabric treated with 0.9 g/m2 4, 4′ DDT was prepared each day and placed on 100% of the wall surface area of the treatment chamber. The mean airborne DDT concentration (denoted by a solid line for each day) was significantly different between days (one way ANOVA; $F = 33.664$; $P < 0.001$). The DDT air concentration measured on Day 3 was significantly higher) than the levels measured on Days 1 and 2 (Holm-Sidak post hoc; $p < 0.001$ for both comparisons). The DDT air concentration measured on Day 1 was significantly higher than the levels measured on Day 2 (Holm-Sidak post hoc; $p = 0.041$).

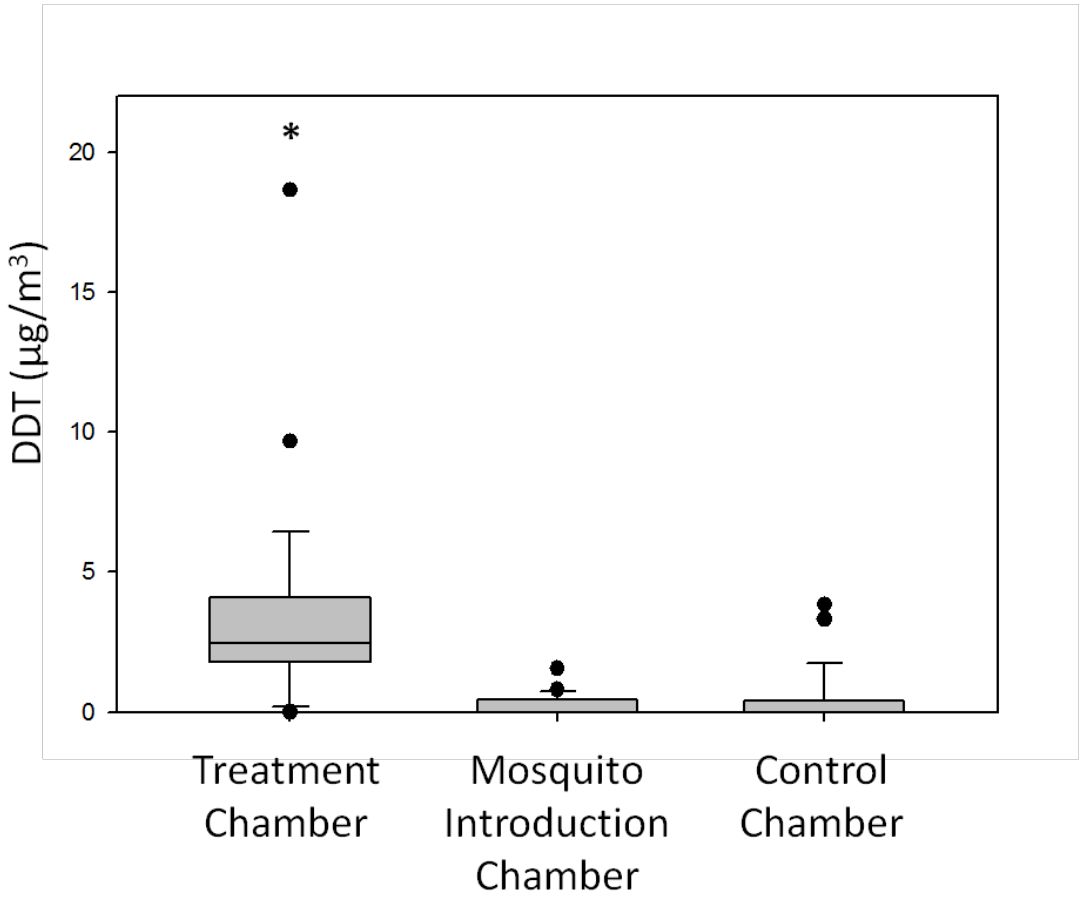

Figure 3-4: Box-and-whisker plot of DDT air concentration in samples collected from the treatment (Fig 1 B), mosquito introduction (Fig 1 C), and control (Fig 1 D) chambers of the laboratory system (black circles denote samples above or below the 90% and 10% percentiles, respectively). Nylon fabric treated with 0.09 g/m2 4, 4′ DDT was prepared each day and placed on 50% of the wall surface area of the treatment chamber. The median airborne DDT concentration was significantly different between days (Kruskal-Wallis one-way ANOVA; $H = 35.461$; $P < 0.001$). The DDT air concentration measured for the treatment chamber was significantly higher than the levels measured in the mosquito introduction and control chambers (Tukey post hoc; $p < 0.05$ for both comparisons).

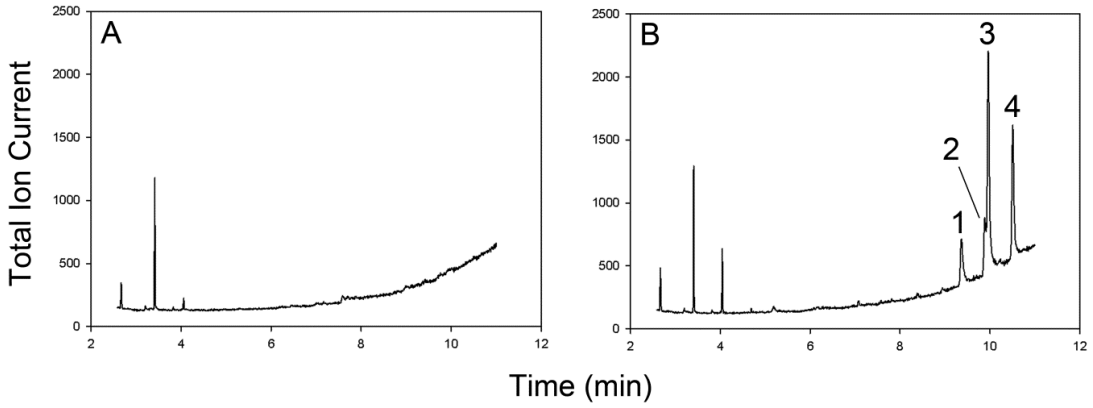

Figure 3-5: Selected ion (m/z 165 and 235) chromatograms for field control hut (A) and treatment hut (B). 4, 4′ DDE (peak 1), 4, 4′ DDD (peak 2), and 2, 4′ DDT (peak 3) were detected with the target analyte 4, 4′ DDT (peak 4). Peak identity was confirmed by retention time and mass spectral data from analytical standards.

TABLES

Table 3-1: Mean DDT air concentrations, with standard deviation in parentheses, determined in the control hut (A) and two treatment huts (B and C).

Hut	Samples Collected	Temperature (°C)	Relative Humidity (%)	$[DDT]_{air}$ ($\mu g/m^3$)	$[DDT] \pm variance_{DDT\ Predicted}$ ($\mu g/m^3$)	Percent quantifiable samples (n)
A	10	25.9 ± 3.3	85.2 ± 11.6	ND*	ND*	10% (1)*
B	17	25.7 ± 3.3	83.5 ± 11.7	0.74 ± 0.45	0.33 – 1.15	64.7% (11)
C	30	25.8 ± 3.0	85.6 ± 13.2	1.42 ± 0.96	1.01 – 1.83	93.3% (28)

*One sample analyzed with 1.22 µg/m3 DDT; a labeling error is suspected

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CHAPTER 4: SIGNIFICANCE AND FUTURE STUDIES

1. SIGNIFICANCE

The research presented here advances the study of spatial repellent (SR) compounds and their characterization in mosquito behavior test systems. Prior to these studies, it was not possible to measure the concentration of active ingredients (AI) during the 10-60 min intervals employed in mosquito behavior experiments. These studies demonstrate that measureable concentrations of airborne semi-volatile AI, e.g., dichlorodiphenyltrichloroethane (DDT) are generated during 10-60 min mosquito behavior assays. The analytical method described in Chapter 2 potentially provides a new tool to identify the lowest concentrations of airborne AI needed to repel mosquitoes during laboratory and field experiments. Ultimately, these experiments may inform policy makers considering inclusion of SR compounds in national and international mosquito control programs.

Evaluation of the concentrations of airborne DDT within these assay systems required the development and validation of a novel sampling and analysis method optimized for short-duration, low-volume sampling. This was accomplished by building on the techniques and protocols previously reported for the study of DDT in water and soil (8), resolution of DDT-related compounds by fast gas chromatography (GC) (2), and determination of airborne pesticide concentrations following agricultural application using small thermal desorption (TD) tubes (3).

This approach represented a departure from previously reported methods for the measurement of concentrations of DDT in the air, which employed sampling intervals of $\geq$ 4 hr, followed by solvent extraction prior to analysis (6; 9-11; 14; 16; 17).

Collectively, the studies reported here are the first to employ TD sample introduction and fast GC resolution of air samples without sample preparation for the determination of concentrations of airborne DDT. By eliminating all sample preparation steps, approximately 18% of the collected sample (~90% extraction efficiency split 5:1 during TD introduction) was introduced into the GC-mass spectrometry (MS) system, ≥ 1,800 times what is possible with traditional methods. In addition to improved sample recovery, the fast GC method employed here allowed baseline resolution of most DDT-related compounds in less than 10 min, with a relatively short cycle time (25 min), compared to traditional methods (≥ 18 h with solvent extraction). High sample throughput (> 50 samples/day) could be realized with the addition of an autosampler capable of unsupervised sample introduction, further improving the utility of this method.

The improvement in sample recovery possible with this TD GC-MS method allowed the determination of airborne DDT concentrations at steady state and within mosquito behavior test systems. The data from these studies validated three key assumptions relevant to determining the concentrations of airborne DDT in test systems and ultimately correlating these measurements with mosquito behavior: 1) DDT is emitted from treated textiles, 2) emission occurred over an operationally relevant temperature range, and 3) significantly different exposure conditions can be generated in laboratory and field mosquito behavior test systems.

Volatilization of DDT has been previously described for soil, glass and organic materials treated with DDT (10; 11; 16; 17); however, this is the first report of DDT emissions from treated textiles. The significantly higher steady state concentrations of airborne DDT observed at 33°C during microchamber experiments were in good

agreement with previous findings reporting substantial increases in vapor pressure (15) and airborne DDT (11). The variation observed in the steady state concentration of airborne DDT over this limited, but operationally relevant temperature range (24°C-33°C), could result in the generation of significantly different exposure conditions despite fixed AI loading and textile surface area conditions. This finding highlights the need to either control the ambient temperature during assays or correct for temperature differences during repeated experiments.

Generating significantly different exposure conditions within a test system is a requirement of any mosquito behavior test system designed to study the repellency of sub-lethal concentrations of airborne AI, e.g., DDT. Data from the evaluations of the laboratory and field test systems verified that significantly different exposure conditions were generated in treatment and control spaces, validating the assumption mosquitoes placed into these systems would be exposed to varied concentrations of airborne DDT. The chemical gradient present in the laboratory (between chambers) and field (between the inside and outside of the experimental huts) systems would allow the study of DDT's repellent effect as mosquitoes could move from areas of high to low concentrations of airborne DDT.

Simultaneously measuring AI concentrations in air and mosquito behavior is critical to identifying exposure conditions capable of eliciting a repellent response in mosquitoes able to transmit disease. The TD GC-MS method developed during this project was applied to the study of mosquito behavior within experimental field huts with and without DDT (1). During this study, mosquito behavior and toxic effects following exposure to DDT were observed on separate groups of female *Aedes aegypti* originating

from the same colony. Two huts containing DDT-treated textiles and a control without DDT-treated textiles were used to evaluate mosquito behavior and knockdown. Volunteers were placed within each of the experimental huts to act as cues for the mosquitoes. During the multi-day trial, mosquito entry into the two huts containing DDT-treated textiles was reduced by 53% and 70% compared to the hut without DDT-treated textiles. Measurable amounts of airborne DDT detected within treated field huts, while mosquitoes were present, confirmed that mosquitoes were exposed to airborne DDT. Furthermore, the lack of observed knock-down or mortality in mosquitoes kept in mesh cages ~1 m above the floor in each hut suggests that sub-lethal concentrations of airborne DDT were sufficient to reduce entry of *Aedes aegypti* mosquitoes into experimental huts containing DDT-treated textiles compared to DDT-free huts.

A previous study of the pesticide resistance status indicates that *Aedes aegypti* strain utilized for the field trials in Thailand was resistant to DDT, with less than half of any tested population sensitive to DDT (mortality range 0% - 37.2%) compared to 100% mortality observed in susceptible strains provide by the U.S. Department of Agriculture (12). The > 50% reduction in entry of resistant mosquitoes suggests that DDT is still an effective repellent at levels not capable of causing observable toxic endpoints, i.e. knock-down or death. This observation is in good agreement with reports suggesting that SR compounds may interact with odor receptors located in the antennae and maxillary palps of mosquitoes (4). The interaction of SR compounds with odor receptors represents a mechanism of action distinct from disruption of nerve impulse conduction responsible for acute toxicity in humans and mosquitoes. This finding suggests mosquitoes may be

repelled regardless of their pesticide resistance status and warrants further investigation with other SR compounds and mosquito strains.

If separate mechanisms of action are responsible for the repellent and toxic actions of DDT, it is critical to determine the concentrations required to illicit a repellent response without inducing morbidity. The TD GC-MS method described here can be extended to future studies to better understand the relationship between exposure (concentration of airborne DDT) and mosquito response. A response curve, with concentration on the x-axis (controlled during experiments) and response, measured as both repellence and knockdown/mortality, on the y-axis, could be developed to identify the lowest concentration needed to repel mosquitoes without knockdown or death. This could lead to the development of a highly efficacious mosquito control program that minimizes the risk of becoming obsolete due to the development of resistance in the target mosquito populations.

2. Future Studies

The methods and findings presented in this work represent the first steps in understanding mosquito behavior following their exposure to sub-lethal concentrations of SR compounds. The two courses of study that naturally follow this work involve identifying the lowest airborne concentration capable of eliciting a repellent response in mosquitoes (of various species) and developing TD GC-MS methods for other semi-volatile compounds to evaluate their SR activity.

Future investigations to find the lowest effective concentration of SR compounds should include the use of known, controllable exposure conditions. The laboratory mosquito behavior assay described in Chapter 3 can be modified to accept air loaded with

SR compounds, confirmed with samples analyzed by TD GC-MS, in place of laboratory supplied air. Commercial air standards generators with the ability to dilute air streams with make-up air and humidity control could be used to vary the concentrations of DDT and relative humidity. These studies are critical for the entomologist to better understand mosquito behavior following exposure to a range of concentrations of airborne DDT at operationally relevant humidity conditions. Additionally, these studies would provide environmental scientists an opportunity to define the impact temperature and relative humidity on the emission rate of DDT and related compounds.

A vital question for public health officials planning vector control campaigns is "how far does the repellent effect extend from one treated house?" The TD GC-MS method could be used to characterize the concentrations of airborne SR compounds at different distances and heights from treated materials. Results from these analyses would inform public health decisions, potentially improving efficacy of vector control programs while reducing cost through evidence-based treatment strategies.

Breaking the mosquito-borne disease transmission cycle will likely require the combined efforts of experts from the fields of vaccine development, virology, field and laboratory entomology, environmental science and public health. The observations of mosquito behavior in the presence of quantifiable amounts of airborne DDT represent a significant initial step in developing tools to evaluate SR compounds and strategies. There are limitations with this first step which must be noted. There are concerns regarding the long term health effects following repeated exposure to sub-acute concentrations of DDT (5; 7), as well as the planned elimination of DDT outlined by the Stockholm Convention (13). The results from these studies should be viewed as a proof

of concept for the application of a TD GC-MS method to measure concentrations of airborne SR compounds.

By applying analytical tools developed for the study of occupational and environmental exposures to entomological experiments, a more complete understanding of sub-lethal exposure conditions on vector behavior may be possible. Understanding how environmental and operational conditions impact the generation rate of SR compounds, e.g., DDT, will be critical to formulation and evaluation of control strategies based on repelling mosquitoes without inducing chemical resistance. The approach described in these studies should be extended to the study of other semi-volatile pesticides capable of exerting SR activity.

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